Exhibit I

Special Issue Article

Development of a laboratory model to assess the removal of biofilm from interproximal spaces by powered tooth brushing

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ABSTRACT: ***Purpose:*** To develop an interproximal laboratory model to compare the potential effectiveness of powered brushing to remove biofilm plaque from interproximal spaces beyond the reach of bristles. ***Materials and Methods:*** *Streptococcus mutans* biofilms were first grown on glass microscope slides in a drip-flow reactor. The slides were removed and positioned in the interproximal model. Each slide was exposed to 15 seconds powered brushing with either the Sonicare Elite or the Braun Oral-B 3D Excel. The thickness of the biofilm was measured with confocal microscopy at various distances from the bristle tips. ***Results:*** The Sonicare Elite reduced the thickness of biofilm by 57% at a distance of 0-5 mm from the bristle tips, 53% at 5-10 mm and 43% at 10-15 mm, relative to biofilm in areas unexposed to brushing. All reductions in thickness were statistically significant ($P < 0.01$). The Braun Oral-B 3D Excel reduced the biofilm thickness by 16%, 13%, and 19% at the same distances respectively, but the thickness reductions were not statistically significant from those in the unexposed areas ($P > 0.1$). (*Am J Dent* 2002;15:12B-17B).

CLINICAL SIGNIFICANCE: The development of a model to assess the effectiveness of powered brushing to remove oral biofilms from interproximal spaces through fluid shear and bubble generation will help evaluate the development and potential ability of powered brushing strategies to control the development of oral biofilms.

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Introduction

Dental plaque biofilms are a complex community of microorganisms that grow on the hard and soft tissues of the oral cavity.[1,2] Routine removal of dental plaque is critical in maintaining oral health, since the uninterrupted growth of plaque biofilms allows the formation of protective niches within which a succession of microorganisms that are the causative agents for caries,[3,4] gingivitis and periodontal disease[5] can proliferate.

The effects of periodontal disease include reversible gingival inflammation and irreversible destruction of periodontal tissues including the gingiva, periodontal ligament, and alveolar bone.[6]

Dental plaque is most commonly removed through routine tooth brushing and flossing.[7] For manual toothbrushes the mechanical removal of dental plaque is achieved primarily through direct contact of toothbrush bristles and the scouring action of bristles across tooth and gum surfaces. However, recently a variety of powered toothbrushes have been developed to improve the efficiency of plaque removal using increased bristle velocity, brush stroke frequency, and various bristle patterns and motions. The Braun Oral-B 3D Excel[a] uses a rotary brush head motion in combination with pulsations along the bristle axis. The Sonicare Elite[b] toothbrush generates direct mechanical brushing and fluid motion by oscillating the bristles up and down from the gingival level to the occlusal surface (Fig. 1).

This study developed an *in vitro* interproximal (IP) model which could be used to evaluate the potential of the mechanical forces generated by powered toothbrushes to reduce the thickness of biofilm beyond the reach of bristles in the interproximal spaces of the teeth both quantitatively and qualitatively. The interproximal model was designed to simulate the exposure of the interproximal plaque between mandibular molar teeth to powered brushing from the buccal surface. Biofilms of *Streptococcus mutans*, an early colonizer of hard tooth surfaces,[8,9] were grown on glass microscope slides in a drip-flow reactor, a simple and inexpensive system used to grow biofilms.[10-12] The colonized slides were then positioned in the interproximal model and exposed for 15 seconds to either the Sonicare Elite or the Braun Oral-B 3D Excel toothbrushes. The biofilm thickness at distances of 0-5, 5-10, and 10-15 mm from the bristles was measured by confocal microscopy and statistically compared with the thickness of biofilm in unexposed areas to estimate percent reduction by brushing.

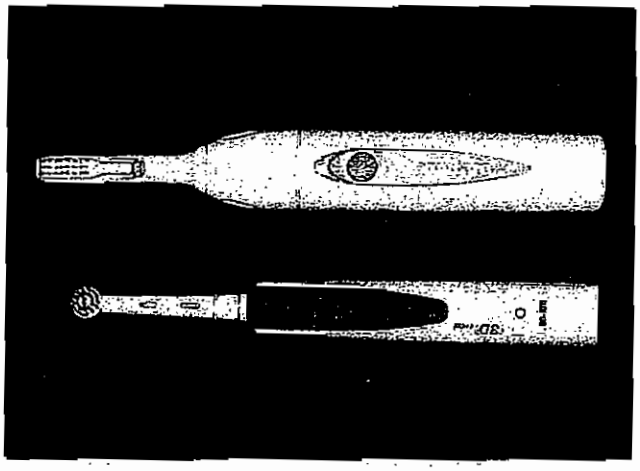

Fig. 1. The Sonicare Elite (top) and the Braun Oral-B 3D Excel (bottom).

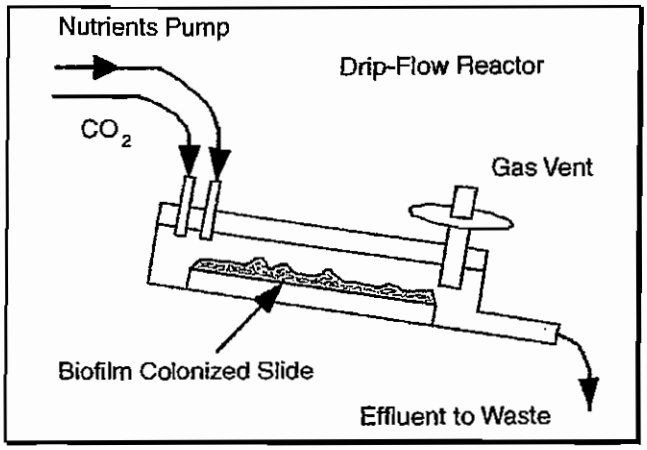

Fig. 2. Schematic of the drip-flow reactor system used to grow the *S. mutans* biofilms. The nutrients were dripped onto the glass slide and flow was gravity driven to the effluent waste line. A CO_2 headspace was maintained in the flow cell.

Materials and Methods

Growth of biofilms

Inoculum culture preparation - Thawed frozen stock culture (100 µl) of *S. mutans* UA159 was added to 100 ml Brain Heart Infusion broth supplemented with 2% sucrose. Cultures were incubated at 35°C overnight in an anaerobic chamber with CO_2 gas generating cartridges. Viable cell density of this culture was determined by serially diluting and drop plating onto solid Brain Heart Infusion agar + 2% sucrose. The plates were incubated overnight in an anaerobic chamber with CO_2 gas generating cartridges.

Drip-flow reactor preparation and sterilization - Biofilms were grown on four glass microscope slides each positioned in a channel of a four channel drip-flow reactor (Fig. 2).[10] Nutrient feed and effluent lines were connected to the reactor with silicon tubing. The entire setup was autoclave sterilized for 20 minutes at 121°C.

Biofilm growth - Approximately 15 ml of Brain Heart Infusion broth + 2% sucrose was added to each of four chambers of a sterile drip-flow reactor. Three ml of an overnight culture of *S. mutans* UA159 was added to each chamber and the channel lids sealed by tightening with Nylon screws. The drip-flow reactor was incubated at 37°C in the presence of CO_2. The reactor was incubated without flow in a level position during for an initial 3-hour period to allow the *S. mutans* cells to attach to the glass slide. After the attachment phase, the drip-flow reactor was placed onto a block with a 10° incline and 1/10 Brain Heart Infusion broth + 2% sucrose media was pumped into each chamber at approximately 0.5 ml/minute for an additional 48 hours (Fig. 2).

Exposure of biofilm to the Sonicare Elite and the Braun Oral-B 3D Excel

Interproximal model - The interproximal model consisted of two acrylic chambers separated by a series of three aluminum posts representing mandibular molar teeth (Fig 3). A biofilm colonized glass microscope slide could be positioned adjacent to the first tooth post by vertically sliding it into place along two lateral guide grooves. The distance representing the interproximal space between the slide and the aluminum tooth post was 1 mm. The depth of the first tooth post was approximately 8.5 mm (7.4 edge-9.9 center mm). A seal was made between the glass slide and the acrylic body with a silicone "O" ring. The slide was held in place by two alligator clips. The chambers were filled with 30 ml of water so that the depth of the water was approximately 10 mm. During brushing the bristles were only partially submerged to simulate saliva coverage in the oral cavity. Both toothbrushes were positioned so that the bristles in the front portion of the toothbrush head were aligned with the interproximal space between the slide and the first artificial tooth and that the furthest extending bristle tips just made contact with the edge of the slide (0 mm bristle distance). According to manufacturer's recommendation, the Braun Oral-B 3D Excel was angled 90° with respect to the artificial dentition and the Sonicare Elite was maintained at a 45° angle.

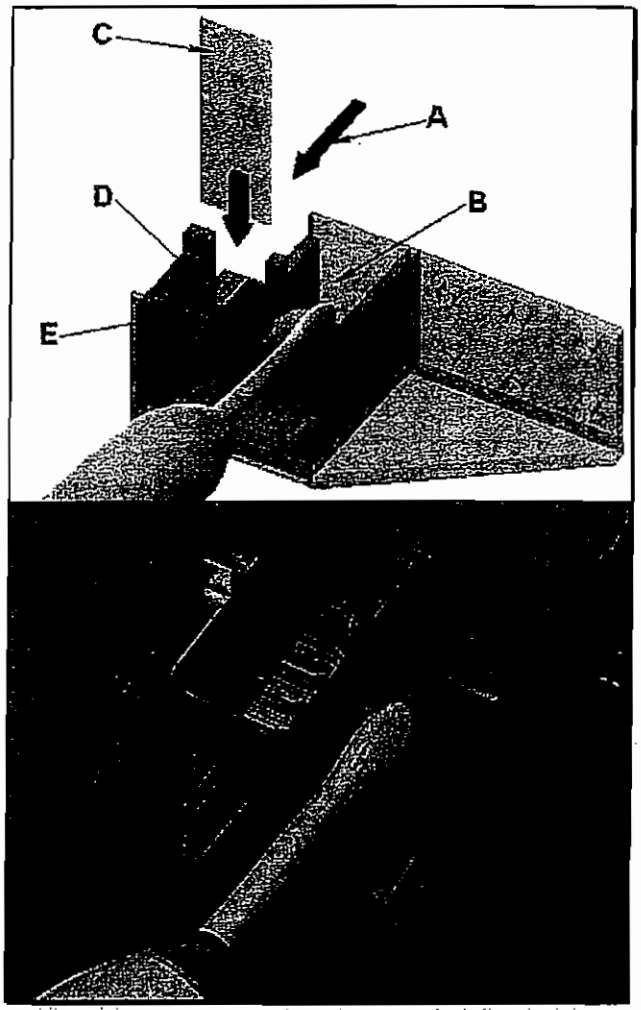

Fig. 3. Solid model drawing and photograph of the interproximal model, the biofilm colonized glass slide and the orientation of the Sonicare Elite toothbrush inserted in the interproximal space between the first tooth post. A) Real time visualization with zoom lens from this side. B) Frontal chamber. C) Microscope slide colonized with biofilm. D) Rear chamber. E) Teeth posts.

Powered brushing in the interproximal model - A biofilm colonized slide was removed from the drip-flow reactor and placed inside a sterile Petri dish for transport. The slide was kept hydrated with sterile Ringers buffering solution. The slide was placed in the interproximal model, and then the model was filled to a depth of approximately 10 mm with sterile Ringers buffer solution. Final adjustments were made to position the

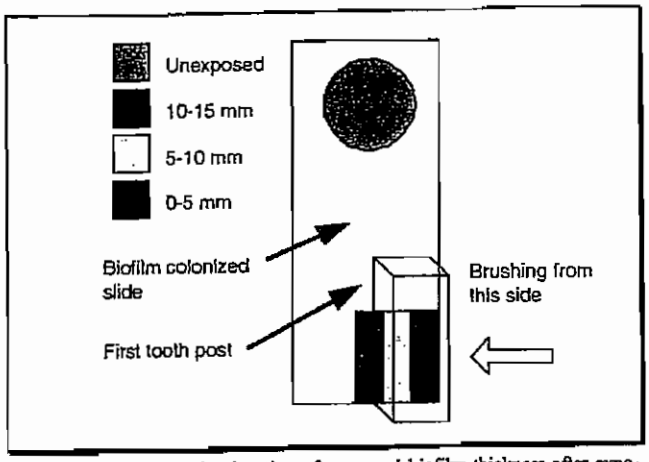

Fig. 4. Schematic showing location of measured biofilm thickness after exposure to 15 seconds powered brushing. Five thickness measurements were taken from the green unexposed area. Three thickness measurements were taken in each of the three other areas at increasing distance from the edge of the slide.

brush head and then the brush was activated for 15 seconds. The slides were removed from the chamber then stained using the Molecular Probes BacLight LIVE/DEAD kit[c] and incubated for 1 hour at 4°C. Live cells in the biofilm stained green and dead cells stained red. The brush and interproximal model were set up prior to the removal of each slide to minimize the time between removal from the growth chamber and brushing.

Biofilm structure and thickness measurements using confocal microscopy - A total of six independent drip-flow reactor growth cycles were used to generate a total of 24 biofilm colonized slides; of these, six were discarded because of visible sloughing of the biofilm or slide breakage during sampling. Two slides from each reactor were exposed to powered brushing from the Braun Oral-B 3D Excel and two slides were exposed to the Sonicare Elite so that in all, nine slides were used for each brush. After brushing and staining, each slide was examined using a Leica TCS-NT confocal microscope.[d] The thickness of the biofilm was measured in four locations on the slide (Fig. 4). Three thickness measurements were made in each of three locations with increasing distance from the bristle tips. Three were taken 0-5 mm from the edge of the slide located closest to the bristles, three at a distance of 5-10 mm, and three at a distance of 10-15 mm (Fig. 4).

In addition, five thickness measurements were made in an unexposed area at the other end of the slide to serve as an internal control. The percent biofilm thickness reduction in each of the exposed interproximal areas relative to the unexposed area for each slide was calculated using the equation:

$$\% \text{ Biofilm Thickness Reduction} = \left(\frac{C - B_i}{C}\right) \cdot 100 \qquad [1]$$

C = Unexposed biofilm thickness;
B = Exposed biofilm thickness; and
i = each exposed region

Statistical analysis - ANOVA between the thicknesses of biofilm in the exposed areas was compared to the thickness in the unexposed area using Minitab[e] (version 13.3). Differences between means were considered significant for $P < 0.01$. The mean values for biofilm thickness did not follow a normal distribution so a log transformation was performed to calculate the Standard Error and perform ANOVA to determine statistical significance between (1) brush types and (2) exposed regions. The ANOVA also calculated Repeatability Standard Deviation (RSD), which measures the repeatability between experiments and individual biofilms. Additionally, univariate ANOVA was performed on the grouped data from all slides to determine if there was a statistically significant difference between the unexposed biofilm thickness and the exposed biofilm thickness for each brush type in each exposed region.

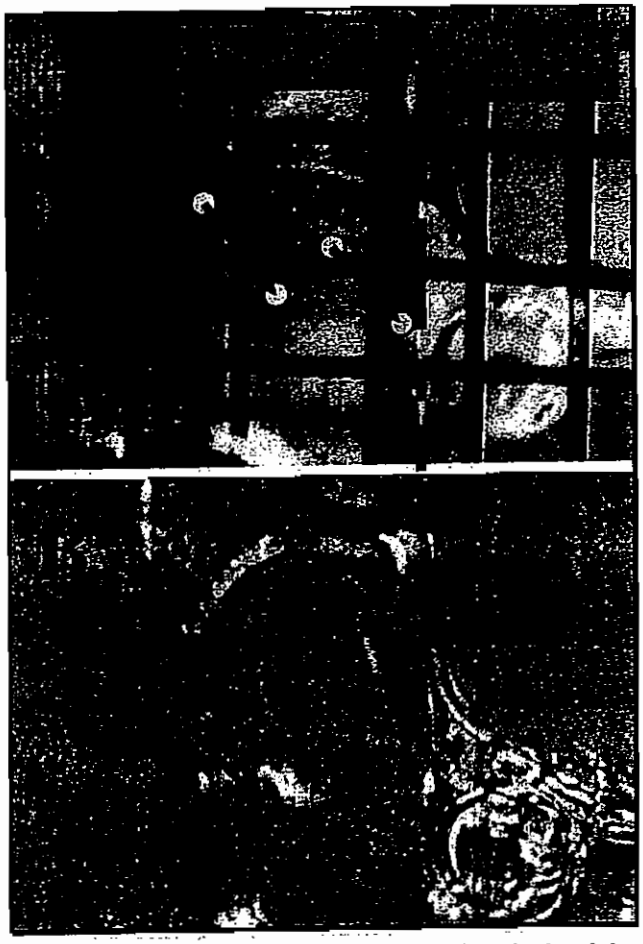

Fig. 5. Flow visualization by tracking the motion of bubbles coming through the interproximal space between the first tooth post and an uncolonized microscope slide positioned in the interproximal chamber. A) Movement of two air bubbles (white and yellow circles) over 1 frame interval (1/60 s). The grid lines were hand drawn (approx. 2.5 mm spacing) on the microscope slide to assist tracking. The red-dashed line indicates the distant edge of the first tooth post, the water level with brush activated is indicated by the blue line. B) The displacement of bubbles over successive video frames were found by subtracting each image from the previous image so bubbles that had moved from a location appeared dark (a) and those that had appeared were light (b). Bubble a-b was traveling at a velocity of 0.14 m/s and the bubble track at "c" gave a velocity of 0.28 m/s.

Flow visualization during powered brushing - The movement of fluid during powered brushing was captured with a Sony power HAD 3CCD[f] color video camera using a 50 mm lens with a x20 zoom. Images were captured on both VHS (60 frames/second) and on a Scion VG-5 PCI[g] framestore board (20 fps) using Scion Image[g] software. Individual bubbles moving through the interproximal space were tracked by Scion Image and image subtraction showed how the position of a bubble changed over successive frames (Fig. 5). The bubble velocity was calculated by measuring the distance traveled over the frame interval.

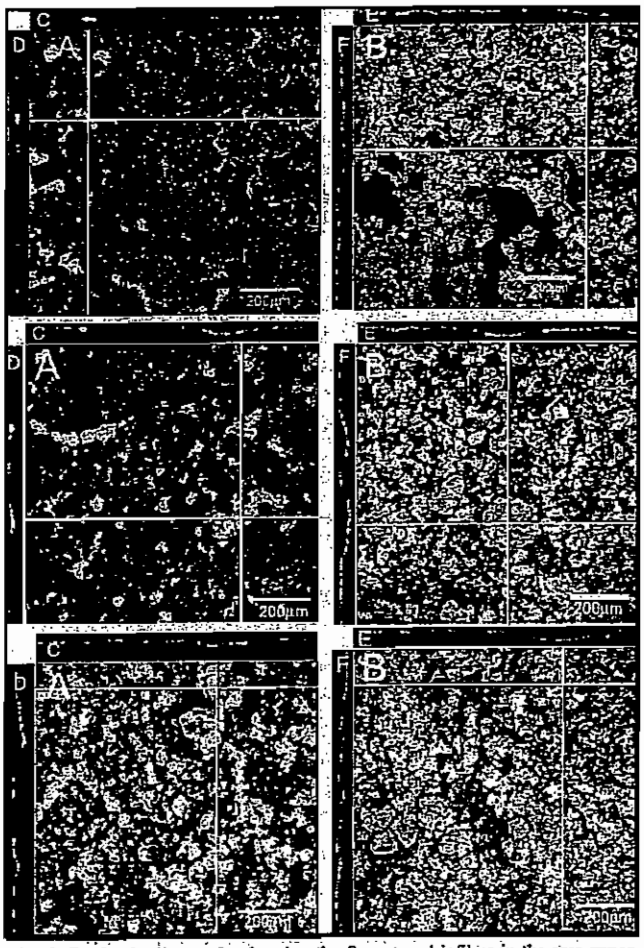

Fig. 6. Confocal micrographs showing the *S. mutans* biofilms in the area zones at distances of 0-5 mm (top), 5-10 mm (middle) and 10-15 mm (bottom) of the slide edge proximal to the bristles with the Sonicare Elite (A) and the Braun Oral-B 3D Excel (B) after 15 seconds powered brushing. Panels CD and EF are cross-sections in locations indicated by white lines. Scale bar = 200 µm.

Results

Biofilm structure - After the 48-hour growth period, *S. mutans* formed an extensive biofilm on the glass slide. The biofilms were very heterogeneous with thickness that ranged from approximately 175 µm to 1,400 µm. Confocal microscopy images revealed that the individual cells in the biofilm were aggregated into cell clusters, which also varied in size from several cells to microcolonies of 200 µm in diameter (Fig. 6). Secondary structures such as towers and channels were clearly visible throughout the biofilm. The thickness of the biofilm in the unexposed region of the slides used for brushing with the Sonicare Elite was 513 ± 258 µm (mean ± 1S.D., n = 45) and 452 ± 277 µm (n = 45) for the Braun Oral-B 3D Excel. There was no significant difference between the "unexposed" thickness in the two slide sets (P= 0.196).

Flow visualization - The Sonicare Elite produced large amounts of bubbles that were projected through the interproximal space between the first tooth post and the glass slide. Bubbles identified in the video sequences were traveling at a velocity of 0.24 ± 0.07 m/s (n=5), with the fastest measured at 0.38 m/s. Water traveling at a similar velocity in a parallel plate flow channel would generate a shear stress on the order of 0.9 Pa. However, many of the bubbles were moving too quickly to be discerned

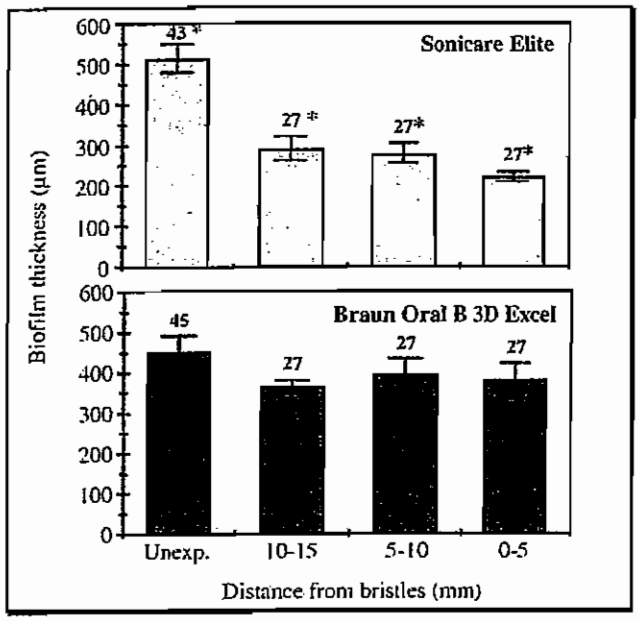

Fig. 7. Mean thickness of *S. mutans* biofilms in each of the exposed areas with decreasing distance from the slide edge in comparison to the unexposed area (unexp.). Bars are 1 standard error. Sample size n is shown above bar. Significant differences compared to the thickness in the unexposed area ($P < 0.01$) are indicated by "*".

in consecutive frames so that the bubble velocities reported here are likely to be an underestimate of the maximum velocity range. During some tests the visible removal of biofilm in patches of up to 1 cm^2 was observed while during other tests removal during brushing was less apparent but confirmed by the appearance of small pieces of detached biofilm in the interproximal water chambers (movies of this process can be viewed at www.erc.montana.edu/Res-Lib99-SW/Movies/default.htm). Bubbles were also generated by the Braun Oral-B 3D Excel but to a lesser extent. These bubbles were traveling at a velocity of 0.20 ± 0.03 m/s (n=5), which was not significantly different from the Sonicare Elite (P= 0.36, n=10). The fastest measured bubble was traveling at a velocity of 0.23 m/second. Fluid traveling at this velocity through the IP space would generate a corresponding shear stress of approximately 0.5 Pa. The removal of large pieces of biofilm was not seen during brushing for any of the nine slides exposed to the Braun Oral-B 3D Excel but detached clumps of biofilm were observed floating in the interproximal water chamber.

Influence of interproximal brushing on biofilm structure - Confocal examination of the biofilms after 15 seconds exposure to powered brushing with the Sonicare Elite showed that a substantial amount of biofilm, which in the unexposed area almost completely covered the slide, was removed, revealing large areas of the underlying glass slide (Fig. 6). The removal was greatest in the area closest to the bristles (0-5 mm) and differences between the biofilm in the unexposed area and the area at a distance of 10-15 mm from the bristles were more difficult to see. There was also biofilm removal by the Braun Oral-B 3D Excel, although not as extensive as the Sonicare Elite (Fig. 6).

There was no discernable difference in the distribution of red and green cells in the biofilm between the unexposed biofilm and the remaining biofilm exposed to either brush type, indicating that the main mode of action of the brushing on the reduction of viable biofilm cells was through physical removal.

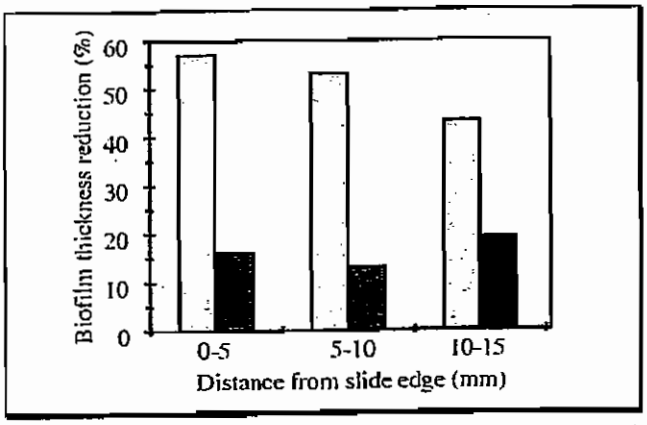

Fig. 8. Percent thickness reduction in *S. mutans* biofilm in each of the exposed areas with increasing distance from the bristle tips for the Sonicare Elite (yellow) and the Braun Oral-B 3D Excel (blue).

Influence of interproximal brushing on biofilm thickness - The quantification of detachment of the biofilm was estimated by measuring the thickness in the exposed and unexposed areas (Fig. 7). For both brushes the thickness of the biofilm was reduced in all of the measured areas in the vicinity of the interproximal space with respect to the thickness in the unexposed area. For the Sonicare Elite the thickness of the biofilm increased with distance away from the bristles. The greatest thickness reduction occurred in the area 0-5 mm from the bristles, which was 221 ± 84 μm (compared with a thickness of 513 ± 257 μm in the unexposed area). The Braun Oral-B 3D Excel did not show this trend and the biofilm thickness was similar in each of the three exposed regions. The thinnest biofilms occurred in the region 10-15 mm from the bristle tips with a thickness of 364 ± 105 μm (compared with a thickness of 452 ± 277 μm in the unexposed area). The differences in thickness in each of the three interproximal areas in relation to the unexposed area was statistically significant for the Sonicare Elite ($P < 0.001$) but not for the Braun Oral-B 3D Excel ($P > 0.109$).

The Sonicare Elite caused a thickness reduction of 57% in the area 0-5 mm from the bristle tips, 53% at 5-10 mm and 43% at 10-15 mm. This compared to reductions of 16%, 13% and 19% respectively for the Braun Oral-B 3D Excel (Fig. 8).

Statistical analysis - The variance between experiments performed on separate days was 0.006. The variance between individual biofilms was 0.041. Using these values, the Repeatability Standard Deviation (RSD) was 0.218 with 13% of the overall experimental variability attributed to experiments done on different days and 87% of the overall experimental variability attributed to individual biofilms. ANOVA showed that there was a significant difference between the biofilm thickness reduction obtained by the Sonicare Elite and that obtained by the Braun Oral-B 3D Excel ($P < 0.01$). There was a slight, but unexplained, difference in thickness reduction ($P = 0.045$) associated with biofilm grown on different days.

Discussion

Biofilm growth - The drip-flow reactor generated extensive *S. mutans* biofilms up to 1.5 mm thick after a 3-hour attachment period and 48-hour growth period. The drip-flow reactor provides an economical and easily operated system for growing biofilms. It is particularly well suited for use as an *in vitro* dental model for the colonization of tooth surfaces since it provides a hard surface which is continually bathed in a thin film (< 0.5 mm) of nutrients and contains a headspace where CO_2 can be easily introduced. The thickness and heterogeneity of the biofilms suggests that localized anoxic regions would develop in the biofilm[13] demonstrating the potential of this system to cultivate more complex mixed community dental biofilms containing both aerobic and anaerobic organisms. The RSD showed that only 13% of the standard deviation was associated with day-to-day variability. This means that there was little error due to the differences in reactor setup or in human inaccuracies in biofilm thickness measurements. The majority of the variability, 87%, could, therefore, be attributed to the natural heterogeneity of the biofilm. However, the large variability in biofilm thickness, while possibly more representative of *in vivo* biofilms, is not necessarily ideal for comparative testing. Biofilm variability could possibly be reduced in future studies by decreasing the attachment and growth periods.

Interproximal model - The interproximal model integrated well with the drip-flow reactor because biofilms grown on the microscope slide could easily be transferred to the interproximal model and positioned in a manner approximating that of biofilm colonizing the interproximal spaces of the oral cavity. However, the thickness of the biofilm (measured in the unexposed areas of the slides used for both Sonicare Elite and Braun Oral-B 3D Excel tests) was highly variable ranging from 150 μm to 1413 μm with a mean of 482 μm (n=88). The standard deviation was 56% (270 μm) of the mean. The mean thickness of the biofilms used for the Sonicare Elite tests (513 μm) was 13% greater than the mean thickness of the biofilms used for the Braun Oral-B 3D Excel tests (452 μm), but this difference was not statistically significant. However, for comparative purposes it is desirable to reduce variability as much as possible, and although naturally grown biofilms often tend to be inherently heterogeneous,[14] reduction of the attachment and growth phases may also reduce thickness and, therefore, variability. An advantage of the more complicated constant-depth film fermenter (CDFF), which has also been used to grow oral biofilms,[15] is that thickness is tightly controlled by mechanically scraping off the top of the biofilm to a predetermined level. Additionally, for the present study glass slides were used so that the flow patterns could be monitored in real time by observation through the back of the slide, but for future studies hydroxyapatite coated slides could easily be used to provide a surface more similar to tooth enamel.

Flow visualization indicated that the Sonicare Elite produced more bubbles than the Braun Oral-B 3D Excel, but the camera frame capture rate of 60 fps was too slow to provide reliable quantification of numbers of bubbles generated and the velocities of the faster bubbles generated by the Sonicare Elite. However, image subtraction did provide an estimate of the flow velocity of the bubbles through the interproximal space. Although faster moving bubbles were seen with Sonicare Elite, the measured velocity was not statistically significant from the Braun Oral-B 3D Excel. However, the motion of bubbles through the simulated interproximal space was clearly visible and for future studies, capturing bubble motion with a high-speed camera may allow better tracking quantification and statistical comparison.

Reduction of biofilm thickness in the interproximal space by powered brushing - Confocal microscopy clearly showed the in-

fluence of powered brushing on the structure and thickness of biofilms. The trends reported here parallel those reported by Hope & Wilson[15] using a different model of the interproximal space. In their model, biofilms were grown on hydroxyapatite disks in a constant-depth film fermenter. They also found the Sonicare Elite toothbrush to remove significantly more biofilm than the Braun Oral-B 3D toothbrush (32% *vs.* 9.5%, P= 0.012).

The effect of biofilm thickness reduction by the Sonicare Elite decreased with increasing distance away from the bristles as expected due to the dissipation of the turbulence by the fluid viscosity. However, even at 10-15 mm away from the bristle tips the thickness of the biofilm was still significantly reduced by 43%. This is relevant since interproximal spaces *in vivo* rarely exceed 10 mm. This trend complements previous studies in which viable cell counts were used to quantify the removal of biofilm by the original Sonicare Advanced toothbrush.[16,17] Those studies report a greater than 60% reduction in *S. mutans* cells adhering to titanium at a distance of 4 mm from the bristle tips[17] and nearly an 80% removal of human dental plaque at a 3 mm distance,[16] both after 15 seconds of powered brushing. The Braun Oral-B 3D Excel also caused a reduction in biofilm thickness but only between a quarter to a half that caused by the Sonicare Elite. The increased biofilm reduction by the Sonicare Elite suggests that this brush produced greater mechanical forces in the interproximal space. This is consistent with a previous study[18] reporting that a Sonicare powered brush produced significantly more force in the interproximal space than the Braun P35 in an *in vitro* model.

In addition to the fluid shear and normal forces created by the bristle motion, both brushes produced air bubbles due to a "beating" effect of the semi-submerged bristles. These bubbles were then forced through the interproximal space. The passage of air bubbles across a biofilm colonized substratum has been shown to remove up to 91% of cells on a conditioned glass surface.[19] The same study also reported that detachment increased at lower bubble velocities because of the greater time allowed to develop the tri-phasic interface between the bubble, the fluid and the solid substratum. However, this detachment was from a sparse covering of recently attached cells in a monolayer, not the type of thick mature biofilm we used in our study. Additionally, it appears that the Sonicare Elite produces numerous bubbles over a wide range of sizes and traveling at a wide range of velocities. The role of bubbles on the detachment of mature biofilms could be investigated in a similar manner to that used by Gómez-Suárez *et al.*[19] Biofilms grown in a parallel plate reactor could be exposed to increased fluid velocity (and therefore, shear) alone and also to the same shear but with introduced bubbles to find the relative contribution to bubble scouring and fluid shear on the detachment of oral-biofilms. However, the present study clearly demonstrated that the Sonicare Elite caused a significant reduction in biofilm thickness in an *in vitro* interproximal space at distances of over 10 mm from the bristles. It is expected that comparable mechanical forces applied *in vivo* would result in a similar effect. Further understanding on the role of mechanical forces on the detachment of biofilms from interproximal spaces and periodontal pockets will further enhance the design of powered toothbrushes to facilitate improved oral health care.

a. Braun GmbH, Kronberg/Ts., Germany.
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d. Leica Microsystems, Wetzlar, Germany.
e. Minitab, Inc., State College, PA, USA.
f. Sony Corporation, Tokyo, Japan.
g. Scion, Inc., Frederick, MD, USA.

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Exhibit J

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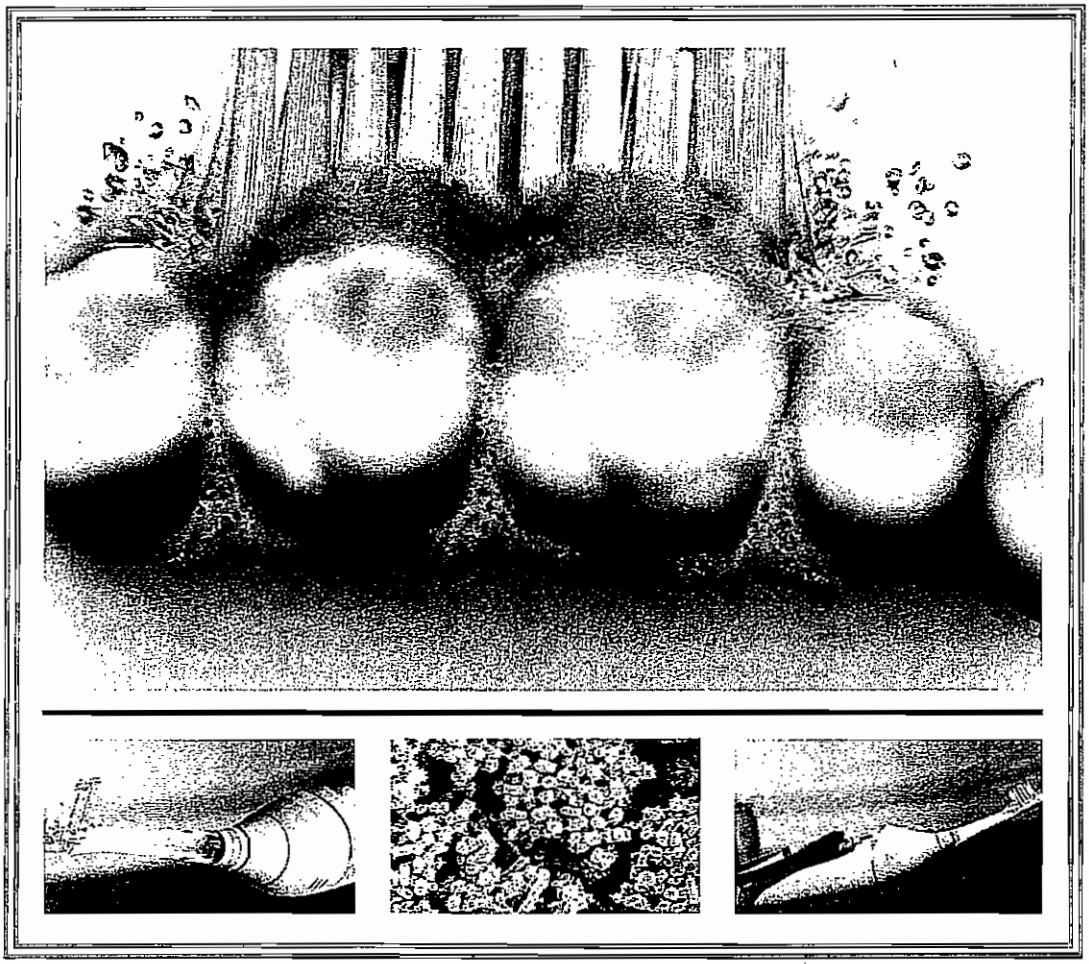
A Supplement to

Compendium

of Continuing Education in Dentistry®

Review of Clinical Research on the IntelliClean System From Sonicare® and Crest®



Supported by Philips Oral Healthcare, Inc, and The Procter & Gamble Company

In vitro Evaluation of the Efficacy and Safety of the IntelliClean System: Interproximal Biofilm Removal and Dentin Substrate Wear

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Abstract: *The ability of a novel integrated power toothbrush and liquid-toothpaste dispensing system, the IntelliClean System from Sonicare® and Crest®, to remove interproximal biofilm while being gentle on dentin was studied using two in vitro models. Interproximal biofilm removal was assessed via a complex multispecies biofilm grown on hydroxyapatite disks and then placed interproximally in a typodont section modeling a typical oral environment. The power toothbrush in the prototype integrated system was compared to a traditional rotating/oscillating power toothbrush, the Oral-B® ProfessionalCare 7000, and a nonbrushing control through a series of 3 experiments with a total of 36 replicates per arm. The amount of interproximal plaque biofilm removed by the integrated system toothbrush was significantly greater than that removed by the rotating/oscillating toothbrush and by the nonbrushing control ($P < .05$). In the second model, dentin substrate wear was measured using profilometry after the brushing of dentin sections (3 mm x 10 mm) for a period equivalent to 2 years of typical product use. Dentin wear associated with the use of the prototype integrated system with standard and whitening versions of the liquid toothpaste was compared to that of a rotating/oscillating power toothbrush and a manual toothbrush with the standard version of the prototype liquid toothpaste, with a total of 12 replicates per arm. The amount of dentin wear induced by the integrated system with either the standard or whitening liquid toothpaste was significantly less than the wear from the rotating/oscillating power toothbrush and the manual toothbrush with the standard liquid toothpaste ($P < .05$).*

The health of the oral cavity relies primarily on diligent oral hygiene. The number of oral hygiene products available to the public has been increasing rapidly in recent years. Elucidation of product efficacy and safety is needed as technological advances result in more effective oral hygiene devices. Dental professionals and consumers need data to make informed decisions and thus requite adequate support for a product's ability to maintain oral hygiene while being safe for extended use. Additionally, comparative information to alternative products allows the user to choose the product best suited to meet specific oral hygiene needs.

The power toothbrush category of oral hygiene products has experienced a tremendous surge in options available to the consumer. The introduction of the Sonicare®,[a] in 1992 and the Sonicare® Elite®,[a] in 2002 have brought innovative features and bristle motions to power toothbrushes. These innovations

[a]Philips Oral Healthcare, Inc, Snoqualmie, WA 98065; 800-676-SONIC

have led to significant improvements in oral hygiene.[1-4] Target objectives achieved with Sonicare® Elite® included increased cleaning efficacy, better access to hard-to-reach areas, and improved user experience through ergonomic design and a smarter feature set.[5] As sonic toothbrush technology evolves and builds on previous accomplishments, a new generation emerges that continues to target all areas of cleaning in the oral cavity—both easy-to-reach and hard-to-reach plaque. The IntelliClean System from Sonicare® and Crest®,[b] integrates an advanced sonic toothbrush technology and a liquid-toothpaste dispensing system to target interproximal plaque.

Early studies provided evidence that fluid forces induced by Sonicare® toothbrushes remove oral biofilm from in vitro dental surfaces.[6-8] As methodology developed, the biofilm was placed in the interproximal space of a typodont model to specifically assess dental-plaque removal efficacy in areas ordinarily inaccessible to the contact of toothbrush bristles.[9] In two subsequent studies, it was determined that the fluid forces associated with the Sonicare® toothbrushes removed more interproximal biofilm than toothbrushes using the rotating/oscillating technology of the Braun Oral-B® 3D Plaque Remover pulsating toothbrush[c].[10,11] The patented range of bristle-tip velocities associated with the amplitude and frequency of bristle-tip motion generates the Sonicare® toothbrush's hydrodynamic fluid forces and is maintained within the IntelliClean System (data on file, Philips Oral Healthcare, Inc).

Elucidation of product efficacy and safety is needed as technological advances result in more effective oral hygiene devices.

Safety also is a concern with any oral hygiene regimen. The wear of exposed dentin as a result of toothbrush and toothpaste use is a well-established area of concern, whereas the wear of enamel and dental materials is a lesser concern.[12-15] As the IntelliClean System is a combination of new brushing technology and new chemistry, it is important to show not only its efficacy but also to consider its safety as an oral care product. Because toothbrushing with toothpaste is the most common method of plaque and extrinsic-stain removal,[16] it is appropriate to test both products together for safety. Previous research has indicated that the Sonicare® toothbrush, when tested with a common toothpaste, results in less dentin-abrasion wear than both an oscillating/rotating power toothbrush and a manual toothbrush.[17]

The purpose of the current in vitro studies was to investigate the ability of a prototype integrated system both to reduce interproximal dental-plaque biofilm and to remain gentle on dentin. These studies were accomplished by using in vitro models and methods closely related to those previously published for evaluating these attributes in power toothbrushes.[10,17]

Biofilm Reduction Experiments

Materials and Methods

The biofilm reduction tests conducted closely followed the methodology employed by Hope and Wilson.[10] This methodology was developed to assess the ability of fluid-induced forces associated with power toothbrushes to cause biofilm removal from interproximal surfaces beyond bristle contact. The constant depth film fermenter (CDFF) has been shown to produce steady-state oral biofilm communities and plaque structures similar to those occurring in the mouth.[18,19] Application of the CDFF biofilm to the in vitro model described by Hope and Wilson provides a measure of the ability of an oral hygiene device to remove biofilm beyond the reach of the bristles. A brief summary of the methodology is provided below.

Treatment Arms

Three treatment arms were used in this test: the prototype integrated system toothbrush as the test device, the Oral-B® ProfessionalCare 7000[c] as a comparative rotating/oscillating power toothbrush, and a nonbrushing control. The devices were tested in the absence of toothpaste, as the components within the toothpaste could influence bacterial viability. Previous studies have shown that inactive power toothbrushes (toothbrush turned off) remove relatively little biofilm bacteria interproximally,[9,10] and thus inactive toothbrush treatment arms were not included in this study.

[b]The Procter & Gamble Co, Cincinnati, OH 45202; 800-492-7378
[c]Oral-B Laboratories, Boston, MA 02127; 800-446-7252

Specimen Preparation

Saliva samples collected from 20 individuals (ranging in age from 20 to 40 years and in good oral health) were processed and stored for use in inoculating the CDFF chambers. The CDFF chambers contained 75 hydroxyapatite (HA) disks[d] on which the biofilm was grown at a constant depth (200 µm). The biofilm was allowed to develop for 4 to 5 days of growth under aerobic conditions. Upon harvest, all HA disks from the CDFF were used in one run of the brushing experiment. A pan containing disks was removed as needed and immediately taken to the brushing apparatus, where the HA disks were removed and placed in sterile Ringer's irrigation solution for subsequent testing. Care was taken not to disturb the biofilm when removing the pans or manipulating the HA disks. Before use in the assay, each biofilm-coated HA disk was dipped without agitation in Ringer's irrigation solution to remove nonadherent bacteria.

Comparative information to alternative products allows the user to choose the product best suited to meet specific oral hygiene needs.

Brushing Experiments

Fully charged power toothbrushes were positioned with respect to the typodont tooth section according to instructions within the instruction manual of each product. Separation between the bristles and the interproximally located HA disks was ensured visually to be 1 mm to 2 mm as the active toothbrush was moved across the typodont. A pair of biofilm-coated HA disks were placed in interproximal recesses between representative mandibular molars, one on each side of the interproximal space flush with the surface of the tooth. In this location, the biofilm-coated HA disks represented plaque on the interproximal tooth surface. The exposure chamber was filled with 7 mL of diluted Saliva Substitute™[e] (33% v/v Ringer's) to mimic fluid levels at the dentition during a typical brushing.

Randomization of the treatment order for the biofilm-coated HA disks was performed by colleagues who were blind to the nature of treatments and outcomes. After each brushing (15 seconds for the 5-tooth typodont section), the fluid within the brushing chamber was completely removed using a disposable curved syringe. This fluid was considered to contain the biofilm bacteria removed as a result of fluid-induced forces of the toothbrush. The fluid was transferred to sterile 15-mL conical tubes, vortexed, and bath-sonicated for 2 minutes. The fluid was diluted 1:10 by adding 1 mL of fluid to 9 mL of sterile Ringer's irrigation solution. Aliquots of this dilution were then plated on tryptic soy agar plates supplemented with 5% sheep's blood using a spiral plater[f]. Plates were incubated aerobically at 37°C for 24 hours in 5% carbon dioxide before bacterial colonies were enumerated.

Each run of a CDFF yields 12 replicates per treatment arm. The study consisted of 3 runs of the CDFF for a total of 36 replicates per treatment arm. Colony-forming units (CFUs) were recorded for each of the replicates and normalized to the volume of brushing fluid sampled (CFU/mL) to represent the amount of biofilm removed with that test.

Analysis

The raw data in CFU/mL were transformed by the base-10 logarithm to create a more normal distribution as appropriate for the subsequent analysis of variance (ANOVA). In the ANOVA, log (CFU/mL) was the dependent variable and treatment group was the independent variable. The null hypothesis was that there is no difference in mean log (CFU/mL) between the three treatment arms. The alternative hypothesis was that at least two of the means differ.

Overall differences between treatments were tested with an F statistic at the $\alpha = 0.05$ level of significance. Multiple comparisons were conducted to specify where the differences exist, if so designated by the F test.

Mean CFU/mL per treatment also was calculated on the raw data for comparison of relative effects. The measure of difference between two treatments was calculated as the ratio of the difference in CFU/mL between one treatment and the nontreatment control to the difference in CFU/mL between the second treatment and the nontreatment control.

[d]Clarkson Chromatography Products, Inc, South Williamsport, PA 17702; 570-323-0450

[e]Roxane Laboratories, Inc, Columbus, OH 43216; 800-962-8364

[f]Spiral Biotech, Norwood, MA 02062; 800-554-1620

Table 1—Biofilm Removed From Interproximally Located Hydroxyapatite Disks

Treatment Group	CFU/mL Mean (SD)	Log (CFU/mL) Mean (SD)	Homogeneous Group[†]
No-treatment control	8.29×10^4 (5.52×10^4)	4.81 (0.31)	A
IntelliClean System (integrated system, toothbrush only)	2.37×10^5 (6.10×10^5)	6.36 (0.11)	B
Oral-B® ProfessionalCare 7000 (rotating/oscillating toothbrush)	5.69×10^5 (3.23×10^5)	5.68 (0.28)	C

*n = 36 each treatment arm.
†Groups in the same column are not statistically different at $P < .05$.
CFU = colony-forming units; SD = standard deviation.

Results

The results of the biofilm removal tests are shown in Table 1 and expressed in CFU/mL and log (CFU/mL). Each treatment was statistically different from the others ($P < .05$). The integrated system toothbrush removed the greatest amount of biofilm from the disk surfaces, 3.7 times more than the rotating/oscillating toothbrush based on the mean number of viable bacteria CFUs removed by each brush.

Dentin-Abrasion Experiments

Materials and Methods

The dentin-abrasion tests conducted closely followed the methodology employed by Sorensen and Nguyen.[17] This methodology was developed specifically for investigating dentin wear associated with the use of power and manual toothbrushes. It takes into consideration the bristle motion inherent in the operation of the toothbrush (eg, movement of the bristles by the toothbrush itself) as well as bristle motion resulting from the user (eg, movement of the toothbrush throughout the mouth). Furthermore, the methodology ensures accurate comparatives such that the dentin is exposed to the complete brush head for the same duration of exposure for all products. A brief summary of the methodology is provided below.

Treatment Arms

As outlined in Table 2, 4 treatment arms were included in the study with chosen parameters for device, toothpaste, and brush-head load. Devices consisted of the integrated system toothbrush as the test device, the Oral-B® ProfessionalCare 7000 as the comparative oscillating/rotating power toothbrush, and the Oral-B® Indicator® 35 manual toothbrush[c] as the control. The integrated system was tested with both the prototype standard and whitening liquid toothpastes; the comparative power toothbrush and the manual toothbrush were tested with only the prototype standard liquid toothpaste. As was described by Sorensen and Nguyen, different loads were used for each device based on available evidence of typical use patterns for these different toothbrush technologies.[17] Each arm consisted of a total of 12 specimens being exposed to treatment, randomized in blocks of 4 (the brushing machine simultaneously brushed 4 specimens of 1 treatment type).

Specimen Preparation and Brushing Experiments

Human dentin was sectioned from extracted teeth using a slow-speed saw with a diamond-wafering blade in a water bath, with cuts made to avoid pulp canals and chambers. The dentin sections were cut to 3 mm by 10 mm and embedded in a resin mold shaped to approximate the curvature of teeth. The surface (dentin section and specimen face) was polished flat, with the exception of the valleys between the representative tooth ridges. Specimens were scanned with a profilometer to

Table 2—Treatment Groups for Dentin Abrasion Study

Group	Toothbrush	Toothpaste	Brush-head Load (g)
1	IntelliClean System (integrated system)	IntelliClean	90
2	IntelliClean System	IntelliClean plus Whitening	90
3	Oral-B® ProfessionalCare 7000 (rotating/oscillating)	IntelliClean	150
4	Oral-B® Indicator® 35 (manual)	IntelliClean	250

Table 3—Average Depth of Toothbrush/Toothpaste-induced Dentin Substrate Wear (µm)

Treatment Group*	Toothpaste	Average Depth (Mean [SD])	Homogeneous Group†
IntelliClean System (integrated system)	IntelliClean	28.0 (7.2)	A
IntelliClean System	IntelliClean plus Whitening	40.6 (14.1)	A
Oral-B® ProfessionalCare 7000 (rotating/oscillating)	IntelliClean	80.1 (27.8)	B
Oral-B® Indicator® 35 (manual)	IntelliClean	67.8 (17.0)	B

*n = 12 each treatment arm.
†Groups in the same column are not statistically different at $P < .05$.
SD = standard deviation.

ensure a smooth surface and were kept hydrated before use. The specimens were randomly assigned to toothbrush treatment groups.

All power toothbrush handles were externally powered so that operating characteristics remained consistent throughout the brushing episode without concern of depleting the batteries. The brush-head motion of the externally powered toothbrushes used in the test was verified to match that of the same brushes before they were altered with the external power leads. The brushes were mounted on a brushing machine so that the complete brush head would move back and forth across the dentin section within the specimen at a constant velocity. This avoided an active brush head dwelling over the dentin section as the machine reversed direction. A new brush head was used for each of the 12 replicates within a treatment arm. A slurry of 0.5 part toothpaste, 0.8 part water, and 1 part artificial saliva (by mass) determined by the treatment arm was applied at a controlled drip (50 mL/min).

Brush exposure on the dentin section approximated an equivalent 2-year typical use of two, 2-minute brushings per day. For the power toothbrushes, this resulted in 52 minutes of brushing per dentin-section surface. For the manual toothbrush, 12,500 strokes across the dentin section represented typical use assuming the user's hand brushes at approximately 2 Hz.

Analysis

Wear on the dentin substrate was evaluated using a profilometer. An impartial individual who was blind to the treatment assignment performed the dentin-wear surface analysis. Scans were taken in triplicate, perpendicular to the brushing machine's motion and at a distance of 1 mm apart. Every set of scans contained a control scan of the unbrushed portion on both sides of the wear facet of the specimen, serving as a reference. The average depth and average maximum depth of the three profiling scans were calculated for each sample across the complete dentin section, followed by the calculation of mean depth and mean maximum depth for each condition based on 12 specimens for each condition.

The quantitative data were analyzed with a one-way ANOVA, with wear as the dependent variable and treatment group as the independent variable. The null hypothesis was that there is no difference in mean dentin wear between the four treatment arms. The alternative hypothesis was that at least two of the means differ.

Differences between treatments were tested with an F statistic at the $\alpha = 0.05$ level of significance. Multiple comparisons were conducted to specify where the differences exist, if so designated by the F test.

Table 4—Average Maximum Depth of Toothbrush/Toothpaste-induced Dentin Substrate Wear (µm)

Treatment Group*	Toothpaste	Average Maximum Depth (Mean [SD])	Homogeneous Group†
IntelliClean System (integrated system)	IntelliClean	55.9 (16.8)	A
IntelliClean System	IntelliClean plus Whitening	79.1 (22.1)	A
Oral-B® ProfessionalCare 7000 (rotating/oscillating)	IntelliClean	149.1 (43.7)	B
Oral-B® Indicator® 35 (manual)	IntelliClean	129.2 (23.7)	B

*n = 12 each treatment arm.
†Groups in the same column are not statistically different at $P < .05$.
SD = standard deviation.

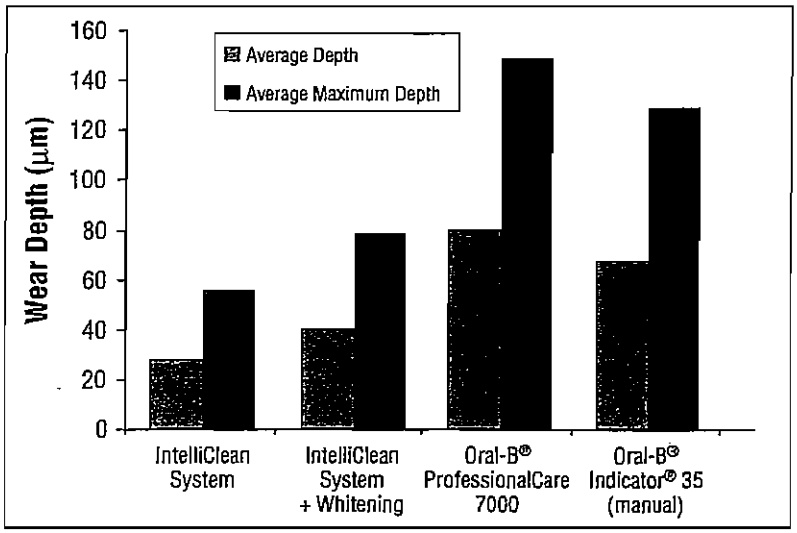

Figure 1—Average and average maximum depths of wear (µm) for each treatment group.

Results

The results of the dentin-abrasion tests can be found in Table 3 (average wear), Table 4 (maximum wear), and Figure 1. For both measures, the two integrated-system treatment groups produced significantly less wear than the rotating/oscillating toothbrush and the manual toothbrush ($P < .05$). There were no significant differences between the two toothpaste arms for the integrated system. Similarly, there were no significant differences between the manual toothbrush and the rotating/oscillating toothbrush.

Discussion

Dental plaque commonly forms on tooth surfaces within the oral cavity. Plaque accumulates preferentially in regions sheltered from the physical forces that may disrupt its adherence. These regions include interproximal spaces, the sulcus, the gumline, and pits and fissures of the tooth structure. Traditionally, the primary means by which a toothbrush cleans the tooth surface is by bristle contact. The direct contact of the bristle with the tooth displaces dental plaque and aids in its removal from the oral cavity. This bristle-to-tooth contact, therefore, limits cleaning to areas directly reached by the bristles. Cleaning and polishing of the tooth surface is aided by toothpaste that may, in part, contribute to wear of the tooth surface, particularly the softer dentin and cementum structures.

In contrast to traditional brushes that rely primarily on direct bristle contact for cleaning, Sonicare® technology has been shown to remove biofilm bacteria beyond the reach of the bristles. The rapid motion of the Sonicare® bristles activates fluid surrounding the brush head, forcing it into regions the bristles do not contact. This fluid motion effects biofilm removal.[7,9-11] Because the rapidly moving bristles function to clean the tooth surface, users typically apply less force than they would with a manual toothbrush.

With respect to the removal of plaque biofilm beyond the reach of the bristles, the current results with the prototype integrated-system toothbrush are consistent with previously published work in which fluid activity associated with the Sonicare® toothbrush was found to remove more biofilm in the interproximal space of this in vitro model than a power toothbrush with rotating/oscillating motion.[10,11] Results presented here demonstrate nearly four times greater removal of biofilm bacteria with the prototype integrated-system toothbrush. This demonstrated superiority is likely a result of the magnitude of the fluid motion generated by the toothbrush and its direction. The toothbrush propels fluid between the teeth as opposed to the rotating/oscillating toothbrush, which generates less fluid motion and generally propels fluid along the smooth surfaces of the teeth (facial or lingual aspect).

Previous studies using this methodology have indicated that this beyond-the-bristles biofilm removal effect was because of the fluid motion generated by the active motion of the bristles (ie, when the bristles were inactive, there was very little removal of biofilm, likely as a result of the minor agitation of fluid as the inactive brush is moved back and forth across the teeth).[10,11] In the current study, a nonbrushing treatment served as a control as opposed to the inactive toothbrush. The biofilm bacteria removed in this control arm represent the spontaneous release of bacteria from the biofilm surface as a result of forces associated with handling the biofilm within a fluid environment. This effect is minimal compared to that of the active prototype or rotating/oscillating toothbrushes.

In the dentin-abrasion study, the prototype integrated system was found to be less abrasive

than the manual toothbrush and the rotating/oscillating toothbrush used with the prototype standard liquid toothpaste. This is consistent with previous results that showed the Sonicare® technology to yield the least amount of dentin wear.[17] No statistical difference was found between the standard and whitening versions of the liquid toothpaste.

The in vitro test methods applied here provide objective analyses of the efficacy and safety of these oral hygiene products. Both the biofilm and dentin-wear experiments have been developed for power toothbrush evaluation, taking into consideration the inherent bristle motion of the device as well as the motion required to move the toothbrush across the tooth surface. They provide methods of assessment of product efficacy representative of what would be found in the oral cavity. These methods build on previous in vitro work within the dental community in which basic models were used to evaluate toothbrush performance[6,8] and dentin wear associated with manual toothbrushes and toothpastes.[20,21]

Conclusion

The IntelliClean System toothbrush, with its associated dynamic fluid activity, demonstrates superior removal of biofilm as compared to a power toothbrush with conventional rotating/oscillating bristle motion. Furthermore, the IntelliClean System with both standard and whitening toothpastes demonstrates significantly lower levels of dentin abrasion than a power toothbrush with rotating/oscillating motion and a manual toothbrush. The results presented here validate that the IntelliClean System is both safe and efficacious and provide the consumer and dental professional with information allowing an informed choice in oral hygiene product selection.

Disclosure

This research was supported by Philips Oral Healthcare, Inc.

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Exhibit K

In Vitro Assessment of the Plaque-Removing Ability of Hydrodynamic Shear Forces Produced Beyond the Bristles by 2 Electric Toothbrushes

Christopher K. Hope,* Aviva Petrie,† and Michael Wilson*

Background: The aim of this study was to compare the efficacy of interproximal plaque removal by the hydrodynamic shear forces produced by 2 electric toothbrushes (toothbrush A, Sonicare Plus and toothbrush B, Braun Oral B) using laboratory-grown plaque biofilms in an anatomically correct in vitro brushing model.

Methods: In vitro oral biofilms were grown in a constant-depth film fermenter on hydroxyapatite (HA) discs. Brushing experiments were conducted in a specially constructed machine designed to simulate the brushing of interproximal plaque between mandibular molar teeth; the bristles of the toothbrush did not make contact with the biofilm at any time. The load force between the brushes and teeth were in accordance with typical use and an exposure time of 5 seconds was used throughout. The efficacy of brushing was assessed by enumeration of the percentage of viable bacteria removed from the HA discs. These experiments were conducted with the electronic effect of the toothbrushes either activated or inactivated.

Results: Activation of toothbrush A significantly ($P = 0.004$) increased the median percentage of bacteria removed from 0.47% to 48.45%. Likewise, activation of toothbrush B significantly ($P = 0.015$) increased the median percentage of bacteria removed from 2.85% to 15.86%. The median percentage of plaque bacteria removed by the active toothbrush A was significantly ($P = 0.009$) greater than that removed by toothbrush B in this model system.

Conclusion: These data imply that toothbrush A would be more effective than toothbrush B at removing interproximal dental plaque in vivo. *J Periodontol 2003;74:1017-1022.*

KEY WORDS

Braun Oral B; comparison studies; dental models; dental plaque/prevention and control; Sonicare Plus; toothbrushing.

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† Biostatistics Unit, Eastman Dental Institute for Oral Health Care Sciences.

Dental plaque is most commonly removed by manual brushing, although a wide range of electrically-powered toothbrushes are now available.[1] The movement of an electric toothbrush head is typically generated by the rotation of an electric motor driving an eccentric cam (for a vibrating action), a series of gears (for a rotary action), or a crankshaft (for an oscillating action). There is evidence to suggest that, in general, plaque removal by electric toothbrushes is better than that achieved by manual brushes.[2] An indirect benefit of using electric toothbrushes is that they often contain timers to give a minimum treatment time and they can be carefully directed to specific areas of the dentition while the electric toothbrush controls the brushing action. The novelty effect, or "gadget appeal," must also be acknowledged with the use of electric toothbrushes, leading to a more disciplined, greater interest in their use.[3]

Toothbrush A‡ (Fig. 1) is purported to remove plaque by direct bristle contact as well as by generating localized hydrodynamic shear forces in fluids surrounding the brush head.[4,5] Toothbrush A operates at a frequency of 260 Hz with a transverse displacement amplitude that varies by applied load, but is typically at

‡ Sonicare Plus, Philips Oral Healthcare Inc., Snoqualmie, WA.

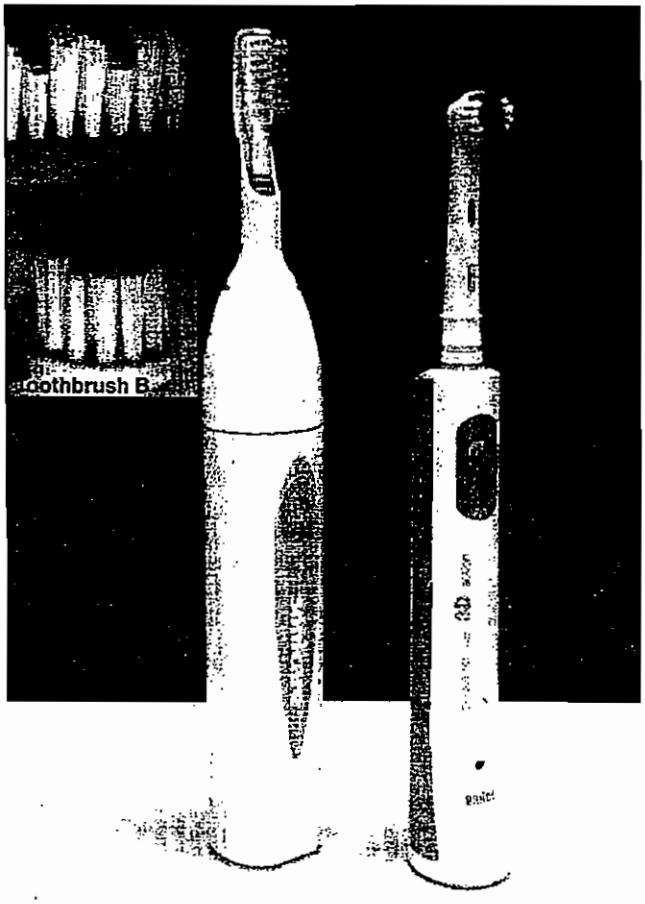

Figure 1.
Toothbrush A (left) and toothbrush B (right) electric toothbrushes. Inset: Brush heads showing the arrangement of bristles.

least 2.5 mm. Microelectronics contained within the brush handle produce a rapidly-oscillating magnetic field, which induces an oscillation in the base of the brush head, causing the head of the brush to resonate. Despite the relatively high energy of the hydrodynamic shear forces generated by toothbrush A, these forces have been shown not to cause any damage to dental or restorative materials.[6] In contrast, toothbrush B§ (Fig. 1) operates by an oscillating/rotating/pulsating head motion. The oscillation frequency is 63 Hz with rotation angle of 56° (approximately 2.8 mm) combined with a perpendicular pulsation operating at a frequency of 170 Hz with an amplitude of approximately 0.15 mm.[7]

Previous in vivo studies have shown the superior ability of toothbrush A in its efficacy of plaque removal and reduction of gingivitis[5,8,9] compared to manual brushing. The superiority of toothbrush B, and its predecessors over manual brushing has also been reported.[10-12]

We have developed a procedure for examining the efficacy of plaque removal by electronic toothbrushes beyond the bristles, which has the advantage of the reproducibility of an in vitro model system married to the realism of a bona fide dental plaque biofilm in an environment similar to the mouth; i.e., the correct spatial arrangement of teeth to simulate interproximal plaque and with available liquid to allow acoustic micro-streaming to occur. In vitro dental plaque biofilms grown in constant-depth film fermenters (CDFF)[13] have been shown to produce steady-state oral biofilm communities similar to those occurring in the mouth.[14] Furthermore, the environmental conditions in the CDFF are more representative of the phase interface conditions present in the mouth than submerged chemostat systems.[15]

The purpose of this study was to quantify and hence compare the effects on in vitro plaque biofilms of fluid shear forces generated by 2 electric toothbrushes with different apparent patterns of fluid activity, toothbrush A and toothbrush B. These effects were studied with a model system representing the interproximal plaque between mandibular molar teeth, brushing from the buccal surface.

MATERIALS AND METHODS

Preparation of CDFF

Biofilms were grown in a CDFF.‖ The CDFF consists of a rotating turntable onto which a nutrient medium/inoculum can be dripped. Scraper blades spread the medium over the sample pans and remove excess biofilm, keeping the biofilms at a constant depth. The CDFF turntable holds 15 polytetrafluoroethylene (PTFE) pans, each of which contains 5 cylindrical holes filled by PTFE plugs. Seventy-five hydroxyapatite (HA) discs¶ were inserted on top of the PTFE plugs and recessed to a depth of 200 µm before sterilization of the CDFF by autoclaving at 121°C for 30 minutes. The contents of the CDFF were exposed to the atmosphere via a 0.2 µm filter.# The operation of the CDFF has undergone an evolution with experience at the Eastman Dental Institute, specifically for growing models of dental plaque.[16-19]

Inoculation of CDFF

A saliva sample was collected from each of 20 individuals (aged 20 to 40 years, in good oral health, a mixture of non-smokers and smokers, male and female) in sterile containers. Five ml of phosphate buffered saline (PBS) were added to each of the samples, which were then pooled together in a sterile beaker containing a magnetic stirrer bar. Glycerol** was added to 15% volume, the mixture was divided

§ Braun Oral B (model D 15), Braun GmbH, Kronberg, Germany.
‖ University of Wales, Cardiff, U.K.
¶ Clarkson Chromatography Products, South Williamsport, PA.
# Hepavent, Whatman, Maidstone, U.K.
** BDH Chemicals, Poole, U.K.

as 2 ml aliquots in cryo-vials and stored at – 80°C. For each inoculation, a thawed 2 ml aliquot from the saliva pool was aseptically added to an inoculation flask containing 1 liter of a complex artificial saliva containing hog gastric mucin without urea[20] at 37°C. The inoculum was then pumped into the sterile CDFF at a rate of 0.5 ml min^{-1}. Artificial saliva medium flow was started concurrently at 0.5 ml min^{-1}, the mean resting saliva flow rate in humans.[21,22]

Biofilm Growth and Sampling

After at least 8 days growth, 3 sampling pans (15 biofilms) were removed from the CDFF. The pans were immediately placed in sterile plastic containers and chilled on ice. Impartiality of pan selection was afforded by removing the PTFE pans from the CDFF in strict numerical order.

Brushing Experiments

The brush to be used during a particular experiment was selected by the drawing of lots. This brush was then charged for 24 hours prior to brushing. In order to measure the separation between the HA discs and the bristles of the toothbrushes, the teeth were marked with a cosmetic lipstick which could easily be removed when contacted by the moving bristles. The brushing machine was set up as described below, but without PBS liquid in the exposure chamber (Fig. 2). PBS was omitted because the aim was to determine whether there was any direct contact between the bristles and the HA discs rather than the extent of any fluid shear activity. The separation distance between disc recess, in the tooth, and the closest approach of the bristle, denoted by removed lipstick, was measured using a calliper with a Vernier scale.

The length of time the brushes were to be activated during these experiments (exposure time) was arrived at by first measuring the distance around the mandibular buccal aspect of an individual (140 mm). Next, the length of the bristle-containing portion of the largest brush head (toothbrush A) was measured (20 mm). The mandibular buccal aspect was therefore 7 "brush heads" in length. Both toothbrushes have a built-in 2-minute timer, with 30 seconds allocated to brush each of the 4 quadrants. This equates to the brush spending approximately 4.3 seconds (30 seconds/7 brush lengths) at a particular point on an aspect. This was rounded up to give the 5 seconds exposure time, used for both toothbrushes, throughout these experiments.

The selection of individual biofilms to be subjected to a particular treatment (i.e., brush activated, brush inactivated, or total count) was performed by colleagues with no vested interest in this project and masked as to the nature of any subsequent treatment. A plastic container holding a sample pan was presented to the biofilm selector, who was asked to make a mark on the exterior of the container, adjacent to one of the 5 biofilms in the sample pan. Subsequent biofilm-covered discs from the pan were selected sequentially in a clockwise direction. Two biofilms were needed for each brushing experiment, representing both sides of the interproximal space.

All brushing experiments were carried out in a class 1 safety cabinet.†† The 2 HA discs were carefully dipped into PBS, without agitation, to remove cells present in the liquid phase above the biofilm proper. These 2 discs were then inserted into recesses located in plastic teeth (Fig. 2 inset) designed to simulate interproximal plaque between mandibular molars, specifically teeth numbers 18 and 19. The teeth containing the discs were in turn placed into an exposure chamber (Fig. 2) mounted on a specially-constructed brushing machine (Fig. 3) via a load cell. The exposure chamber was carefully filled with 7 ml PBS containing 0.8 g l^{-1} hog gastric mucin.‡‡ The angle of the brush heads was 40° with a horizontal and vertical load of 62 ± 5g (88 g vector total) for toothbrush A while toothbrush B was perpendicular to the teeth with a horizontal load of 150 ± 10g and a minimal vertical load. These loads were in accordance with the manufacturers' recommendations based upon typical use. Brushing was conducted from the buccal aspect. The reciprocating brushing action of 0.26 Hz was initiated,

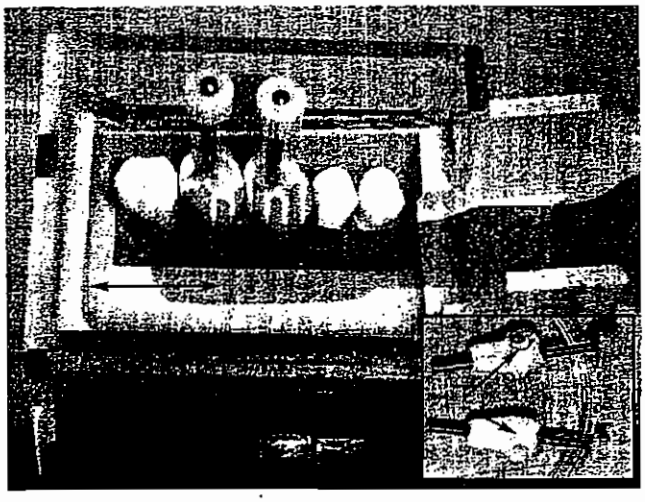

Figure 2.
The exposure chamber (without lid). Brushing is from the buccal surface, the approximate position of the toothbrush is shown as a shadow. The HA discs are held perpendicular to the plane of the brushing action (arrow) and are separated from the bristles by a distance of at least 1.62 mm. Inset: Molars with recesses for locating the 5 mm hydroxyapatite discs shown by arrows.

†† British Standard, BS 5726-1992, Medical Air Technology Ltd., Manchester, U.K.
‡‡ Sigma-Aldrich Co. Ltd., Gillingham, Dorset, U.K.

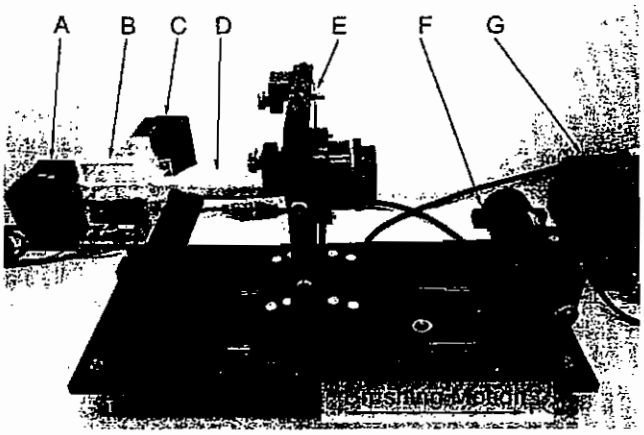

Figure 3.
The brushing machine. A. Transformer. B. Exposure chamber (with lid in place). C. Load cell. D. Electric toothbrush. E. Adjustable toothbrush mounting stage. F. Electric motor with eccentric cam connected to E via a cronkshaft. G. Motor speed controller, normally set to give a brushing frequency of 0.26 Hz.

along with the electronic action of the brush. The distance travelled by the brushes during one cycle was 9.5 mm with an exposure time of 5 seconds. These procedures were then repeated upon similar biofilms for the inactivated (i.e., power off) brushes.

The exposure chamber was disinfected with 70% ethanol between experiments. This was flushed out with sterile PBS after a contact time of 5 minutes.

Post-Treatment Sampling

After the brushing treatment, approximately 5 ml of the brushing solution was transferred from the sample holder into a sterile, plastic container (25 ml volume) along with sterile glass beads. The samples were then vortex-mixed for 30 seconds in order to break up any clumps of bacteria, and serial dilutions were prepared in sterile PBS.

To determine the number of viable cells remaining on the HA discs after a brushing treatment, the 2 treated discs were placed together into 7 ml of brushing solution containing sterile glass beads. These discs were then vortex-mixed for 30 seconds and further serial dilutions were prepared in sterile PBS. Representative biofilm-covered HA discs had previously been examined by scanning electron microscopy after vortex mixing to ensure that all adhered bacteria would be removed by vortex mixing.

The total viable count of bacteria on the discs was established by dipping 2 untreated biofilm discs in 1 ml PBS as described above and then placing the discs directly into 7 ml of brushing solution containing sterile glass beads. These discs were then vortex-mixed for 30 seconds and further serial dilutions prepared in sterile PBS.

Aliquots of the serial dilutions were plated onto blood agar[§§] (BA) to determine the number of colony-forming units (cfu) in each sample. Plating was replicated 12 times for each treatment and dilution to obtain an accurate value of the number of bacteria present in a particular sample. All plates were incubated in an anaerobic environment at 37°C for 72 hours, at which point the mean number of cfu was calculated (n = 12).

The percentage of biofilm removed from the disks was calculated on the basis that the total number of bacteria originally present was equal to the number of bacteria removed plus the number of bacteria remaining, i.e., % removed = bacteria remaining/(bacteria remaining + bacteria removed) × 100%.

The experiments were repeated a total of 23 times using 4 different brushing regimens: 6 with toothbrush A activated; 6 with toothbrush B activated; 5 with toothbrush A inactivated; and 6 with toothbrush B inactivated.

Statistical Analysis

The following *P* values were calculated to measure the significance of the statistical evidence against the null hypotheses: 1) active and inactive toothbrush A removed the same percentage of bacteria; 2) active and inactive toothbrush B removed the same percentage of bacteria; and 3) active toothbrush A and active toothbrush B removed the same percentage of bacteria.

P values below 0.05 were considered significant and led to rejection of the null hypothesis. The non-parametric Mann Whitney U test was performed to compare the median percentage of bacteria removed in the 2 groups.

RESULTS

Lipstick removal patterns showed that the bristles of the 2 brushes did not make contact with the HA disc at any point in the brushing cycle and the mean bristle separation distances for the 2 brushes were 2.65 mm for toothbrush A and 1.62 mm for toothbrush B.

The median percentages of viable bacteria removed by the 2 brushes when activated or inactivated are shown in Table 1.

A Mann-Whitney U test (a non-parametric comparison of the medians of 2 independent samples) showed that, with their electronic effects activated, both toothbrushes removed significantly more plaque bacteria from the biofilms than brushing with their electronic effects inactivated (Toothbrush B; $P = 0.015$, n = 12: toothbrush A; $P = 0.004$, n = 11). The Mann-Whitney U test also showed that the percentage of plaque bacteria removed by toothbrush A beyond the bristles after 5 seconds of exposure was significantly greater than that removed by toothbrush B ($P = 0.009$; n = 12).

§§ Becton Dickinson, Franklin Lakes, NJ.

Table 1.

Median Percentage of Oral Biofilm Removed from HA Discs Enumerated as cfu for Brushing Treatments

Treatment	N	Median	Interquartile Range
Toothbrush A			
Active	6	48.45	30.61-73.81
Inactive	5	0.47	0.37-4.10
Toothbrush B			
Active	6	15.86	10.61-23.65
Inactive	6	2.85	1.78-11.76

The median percentage of plaque removed by the toothbrush A (48.45%) was 3.05 times greater than the toothbrush B (D15) (15.86%).

DISCUSSION

There are conflicting reports of the efficacy of electric toothbrushes in comparison trials of interproximal access and plaque removal.[23] The problems associated with obtaining objective results from clinical trials are well known. Major problems with in vivo studies of plaque removal are the poor reproducibility of results, the inability to standardize treatment, and natural variations in the human oral microflora. Furthermore, in vivo studies are often conducted by members of academic research institutions where the "dental IQ" of patients is relatively high.[24] Another issue with in vivo studies is the patient's personal preference for a particular brush,[25] which could further bias the results from such a clinical trial. To ascertain the actual plaque removal efficacy of different electric toothbrushes at a specific site within the oral cavity, an in vitro brushing system has the advantage that these factors are eliminated. Using such a system, the results of this study have shown that the percentage of plaque bacteria removed by toothbrush A (48.45%) was significantly greater ($P = 0.009$) than that removed by toothbrush B (15.86%).

A clinical comparison of the efficacy of plaque removal by toothbrush A and toothbrush B has also been undertaken.[25] In that study, quadrants of subjects' mouths were randomly assigned treatment with toothbrush A or toothbrush B and changes in plaque coverage, using the Modified Navy Plaque Index (MNPI),[26] were recorded. The results showed that, with respect to the reduction of interproximal plaque scores, toothbrush B achieved an 87% reduction and toothbrush A a 68% reduction ($P = 0.001$). These data are contrary to the results obtained in the present study. A possible explanation is that the MNPI is qualitative; by definition, the index requires that the plaque must be visible to be scored and plaque deep within the interproximal space (up to 5.15 mm from the site of bristle access to the center of the HA disc in our model) could not have been seen. A compounding factor is that the MNPI[26] provides little scope for accurately measuring any reduction in the thickness of the plaque after brushing. Enumerating the number of bacteria removed from plaque biofilms provides a quantitative interrogation of any reduction in the volume of the brushed plaque.

The bristle-tip velocity of the 2 brushes used in the present study can be calculated by the wave-equation: $v = 2\pi fA$, where v = velocity, f = frequency of oscillation, and A = amplitude of oscillation. The peak bristle-tip velocities for the 2 different movements (rotation and perpendicular pulsing) of toothbrush B were summed via vector addition to give a combined bristle-tip velocity. Toothbrush A has a bristle-tip speed of 4.08 m s^{-1}; toothbrush B has a combined bristle-tip speed of 1.12 m s^{-1}. This translates to toothbrush A having a bristle-tip speed 3.64 times greater than toothbrush B. This figure is similar to the ratio of the median percentage of bacteria removed between the 2 brushes; toothbrush A removed 3.05 times that of toothbrush B, suggesting that the bristle-tip velocity may be related to the efficacy of plaque removal beyond the bristles for these 2 toothbrushes.

Previous in vitro studies of the efficacy of electric toothbrushes have provided interesting results but have, in our opinion, used inappropriate models of dental plaque such as those employed by Driesen et al.[27] and Khambay and Walmsley.[4] Driesen et al.[27] used a robotic arm and an "artificial head" to compare the efficacy of toothbrush B with its predecessor. While being an ingenious and extremely elegant system, the experiment involved using a paint mixture to simulate plaque and the brushing system was essentially dry, preventing the elucidation of plaque removal by fluid shear forces. The experiments performed by Khambay and Walmsley[4] evaluated electric toothbrushes in an in vitro environment by measuring the removal of a "plaque" composed of a 1 mm thick film of soft cheese. Brushing was conducted with and without water to assess the efficacy of "plaque" removal due to acoustic microstreaming. Brushing without water was carried out under a 30 g load and brushing with water was carried out with a distance of 1 mm between the bristles and the "plaque." The results of this study did not show a significant difference between the "plaque" removal efficacy of toothbrush A and toothbrush B (model number not specified). The validity of this study however, was undermined by the use of a film of soft cheese and an oversimplified model of brushing.

The saliva pool used to inoculate the CDFF represented an "average" saliva rather the saliva of a single "typical" individual. The most important feature of the saliva pool was that it contained a volume of saliva

(>150 ml) sufficient to provide duplicate inocula for a large number of experiments.

In conclusion, with their electronic effects activated, both Sonicare Plus, toothbrush A (48.45%) and Braun Oral B, toothbrush B (15.86%) removed a significantly higher percentage of interproximal plaque, beyond the bristles, than with their electronic effects inactivated (0.47% and 2.85%, respectively). This shows that the fluid shear forces generated by both toothbrush A and toothbrush B are capable of removing plaque bacteria beyond the bristles. However, toothbrush A removed significantly more plaque beyond the bristles than toothbrush B ($P = 0.009$).

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Funding for this project was provided by Philips Oral Healthcare Inc., Snoqualmie, Washington. The authors thank the staff at the Eastman Dental Institute for Oral Healthcare Sciences, Department of Microbiology, University College London for assisting in the randomization and confirmation policies required by this protocol; Jonathan Knowles, Head of Department, Department of Biomaterials; and Marilou Ciantar, Department of Microbiology, for an informal validation of the brushing machine and dental model, respectively.

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Exhibit L

Special Issue Article

Comparison of the interproximal plaque removal efficacy of two powered toothbrushes using *in vitro* oral biofilms

CHRISTOPHER K. HOPE, PHD & MICHAEL WILSON, DSC

ABSTRACT: ***Purpose:*** To compare the efficacy of interproximal plaque removal beyond the bristles by two electric toothbrushes, the new Sonicare Elite and Braun Oral-B 3D, in an *in vitro* model replicating the brushing of interproximal plaque as would occur *in vivo*. ***Materials and Methods:*** Oral biofilms were grown, *in vitro*, in a constant-depth film fermenter on a hydroxyapatite (HA) disc substratum. Brushing experiments were conducted in a brushing machine representing the brushing of interproximal plaque between mandibular molars. The HA discs with oral biofilms were located a minimum of 1.6 mm away from the bristles. The efficacy of plaque removal was assessed by enumeration of the percentage of viable bacteria removed from the biofilms by 5 seconds of brushing with the brush motors either activated or inactivated. ***Results:*** In the activated state, both brushes removed a significantly higher percentage of plaque bacteria compared to the inactive brushes (Braun, P= 0.002; Sonicare, P= 0.005). The percentage of plaque bacteria removed by the Sonicare Elite (32.23%) beyond the bristles was significantly greater (P= 0.012) than that removed by the Braun Oral-B 3D (9.48%) in this model system. (*Am J Dent* 2002;15:7B-11B).

CLINICAL SIGNIFICANCE: The Sonicare Elite electric toothbrush removes a significantly higher percentage of plaque bacteria beyond the reach of the bristles than a Braun Oral-B 3D in this *in vitro* model of interproximal plaque.

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Introduction

Adequate oral hygiene is an important part of maintaining health in the oral cavity.[1] Consumers have a choice of dental products to assist oral hygiene efforts. Perhaps the most common plaque removal product, at least in Western society, is the toothbrush. Toothbrushes, and most other mechanical oral hygiene products, target dental plaque removal from the tooth surface. Other products, such as antimicrobial mouth rinses, may target plaque development as a way to maintain oral health.[2] Only with proper techniques and products to target plaque removal or development, are individuals able to maintain oral hygiene to a level sufficient to prevent gingivitis and periodontal disease by regular brushing at least once every second day.[3,4]

Both manual and electric-powered toothbrushes are available to assist individual oral hygiene efforts. Manual toothbrushes rely on the user to generate the brush motion as well as guide the brush around the mouth, thereby contacting exposed tooth surface to remove plaque. Powered toothbrushes typically have an electrically powered motor that generates bristle motion, and rely only on the user to guide the moving bristles across the dentition so that all exposed tooth surfaces are contacted. Regardless of brush type, the ability of the user to access as many tooth surfaces as possible aids in plaque removal. However, with some toothbrushes, it is only the exposed tooth surfaces that are contacted by the bristles, leaving areas such as the interproximal region to be cleaned by other aids such as dental floss.

Some oral hygiene products are now marketed with claims of using fluid motion to help access difficult to reach areas of the oral cavity,[5] thus using forces associated with moving fluid to assist in oral biofilm removal.[6] One such product is the Sonicare toothbrush.[a] The ability of hydrodynamic fluid forces associated with the Sonicare to remove oral biofilm from model dental surfaces has been investigated in several *in vitro* studies.[7-9] The Sonicare has been shown in these laboratory studies to remove biofilm created both *in vitro* and *in vivo* (human dental plaque). Although these studies elucidated hydrodynamic effects on biofilm, it was felt that they could be improved upon by investigating biofilms in an orientation in which dental plaque is typically inaccessible to bristle contact, for example, the interproximal space.

A procedure has been developed for investigating the plaque removal efficacy of the hydrodynamic fluid forces associated with powered toothbrushes using a brushing machine that replicates the brushing of interproximal plaque between mandibular molars. Oral biofilms can be inserted into machined recesses between these teeth and subsequently placed into an exposure chamber that can be partially filled with liquid. The position of the brush against the gingival margin, in this model, can be adjusted and the load between the brush and the teeth measured by a load cell. This procedure has the advantage of the reproducibility of an *in vitro* model system combined with the realism of a *bona fide* dental plaque biofilm. The environment for this model simulates the correct spatial arrangement of teeth with available liquid to allow hydrodynamic fluid forces between teeth as may occur in the oral cavity.

In vitro dental plaque biofilms grown in constant-depth film fermenters (CDFF)[10] have been shown to produce steady-state oral biofilm communities and plaque structures similar to those occurring in the mouth.[11,12] Furthermore, the environmental conditions in the CDFF are more representative of the phase interface conditions present in the mouth than submerged chemostat systems.[13]

Powered brushes that generate bristle movement will also generate a certain degree of fluid motion in the oral cavity.

Both the force and direction of fluid motion will contribute to plaque removal, particularly in the interproximal region. The purpose of this study was to use our biofilm model to compare the effects on *in vitro* dental plaque biofilms of fluid forces generated by two electric toothbrushes with different patterns of fluid activity, the Sonicare Elite and the Braun Oral-B 3D.[b] The effect of fluid motion was studied with a model system representing the interproximal plaque between mandibular molar teeth, brushing from the buccal surface.

Materials and Methods

Preparation of the CDFF - Biofilms were grown in a CDFF.[c] The CDFF consists of a rotating turntable onto which a nutrient media/inoculum can be dripped. Scraper blades spread media over the sample pans and remove excess biofilms, keeping them at a constant depth. The CDFF turntable holds 15 polytetrafluoroethylene (PTFE) pans, each pan containing five cylindrical holes filled by PTFE plugs. Seventy-five hydroxyapatite (HA) discs[d] were inserted on top of the PTFE plugs and recessed to a depth of 200 µm before sterilization of the CDFF by autoclaving at 121°C for 30 minutes. The CDFF in this instance was exposed to the atmosphere *via* a 0.2 µm Hepavent filter.[e] The *modus operandi* for the CDFF[10] has undergone an evolution with experience at the Eastman Dental Institute, specifically for growing models of dental plaque.[12,14-17]

Inoculation of the CDFF - Saliva samples were collected from 20 individuals (aged 20-40 years, in good oral health, a mixture of non-smokers and smokers) in sterile containers. Five ml of sterile phosphate buffered saline (PBS) was added to each of the samples and pooled in a sterile beaker containing a magnetic stirrer bar. Glycerol[f] was added to 15% volume, the mixture divided as 2 ml aliquots in cryo-vials and stored at -80°C. For each inoculation, a thawed 2 ml aliquot from the saliva pool was aseptically added to an inoculation flask containing 1 liter of a complex artificial saliva containing hog gastric mucin, without urea[18] at 37°C. The inoculum was then pumped into the sterile CDFF at a rate of 0.5 ml/minute. Artificial saliva medium flow was started concurrently at 0.5 ml/minute (the mean resting saliva flow rate in humans).[19,20] The CDFF was maintained at 37°C under aerobic conditions.

Biofilm growth and sampling - After at least 8 days growth, three sampling pans (15 biofilms) were removed from the CDFF. The pans were immediately placed into sterile plastic universals and chilled on ice. Impartiality of pan selection was afforded by removing the PTFE pans in strict numerical order (the proper operation of the CDFF also requires that sample pans were removed sequentially). Only 6 of the 15 discs in the three pans were needed for an experiment.

Brushing Experiments

Preparation - The brush to be used during a particular experiment was randomly selected and was then charged for 24 hours prior to brushing. In order to ensure the separation between the discs and the bristles of the toothbrushes, the teeth were marked with a cosmetic lipstick which could easily be removed when contacted by the moving bristles. The brushing machine was set up as described below, but without PBS liquid in the sample holder. The PBS was omitted because the aim was to determine whether there was any direct contact between the bristles and the HA discs rather than the extent of any fluid shear activity. The separation distance between the disc recess in the tooth and the closest approach of the bristle, denoted by removed lipstick, was measured using a calliper with a Vernier scale.

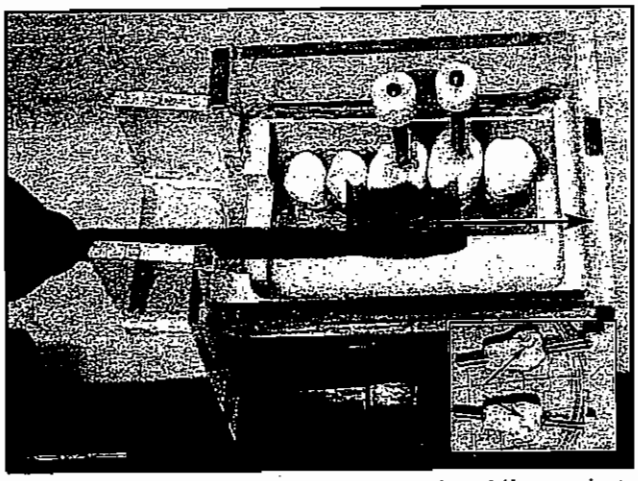

Fig. 1. The exposure chamber with the plastic teeth located (the approximate position of the brush is shown as a shadow) representing mandibular molars. Inset. Plastic teeth containing a recess for a biofilm covered 5-mm HA disc.

Biofilm selection - The selection of individual biofilms to be subjected to a particular treatment (*i.e.* brush activated, brush inactivated or total count) were performed by colleagues who were blind as to the nature of any subsequent treatment.

Brushing - All brushing experiments were carried out in a Class 1 (British Standard; BS 5726-1992) safety cabinet[g] and aseptic technique was observed (Fig. 1). A pair of HA discs were carefully dipped, without agitation, into PBS to remove cells present in the liquid phase above the biofilm proper. The two discs were then inserted into recesses in the interproximal surfaces of plastic teeth.[h] The discs so located simulated interproximal plaque between mandibular molar teeth. The teeth containing the discs were in turn placed with neighboring teeth into an exposure chamber mounted on a specially-constructed brushing machine[a] *via* a load cell. The exposure chamber was filled with 7 ml PBS containing 0.8 g/l hog gastric mucin to simulate fluid levels surrounding the dentition during typical brushing. The reciprocating brushing action of 0.26 Hz was initiated. The brush heads were angled at 40° with a horizontal and vertical load of 62 ± 5 g (88 g vector total) for the Sonicare Elite while the Braun Oral-B 3D was positioned perpendicular to the teeth with a horizontal load of 150 ± 10 g and a minimal vertical load. For each brush, the load was in accordance with its manufacturer's recommendation based upon typical use. The distance traveled by the brushes during one cycle was 9.5 mm with an exposure time of 5 seconds. These procedures were repeated for the inactivated (*i.e.* power off) brushes.

The exposure chamber was disinfected with 70% ethanol between experiments. This was flushed out by sterile PBS after a contact time of 5 minutes.

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Post-treatment sampling. After the brushing treatment, 5 ml of the brushing solution was transferred from the sample holder into a sterile, plastic container with sterile glass beads. The samples were then vortex-mixed for 30 seconds to break up any clumps of bacteria, and serial dilutions were prepared in sterile PBS.

In order to determine the number of viable cells remaining on the hydroxyapatite discs after treatment, the treated discs were placed into 7 ml of brushing solution containing sterile glass beads. These discs were then vortex-mixed for 30 seconds and further serial dilutions prepared in sterile PBS. Representative biofilm covered HA discs had previously been examined by scanning electron microscopy after vortex mixing to ensure that all adhered bacteria would be removed by this technique.

The total viable count of bacteria on the discs was established by dipping untreated discs in 1 ml PBS as described above and then placing the discs in 7 ml brushing solution containing sterile glass beads. These discs were then vortex-mixed for 30 seeonds and further serial dilutions prepared in sterile PBS.

Aliquots of the serial dilutions were plated onto blood agar[i] (BA) for total anaerobic viable counts. Plating was replieated 12 times for each treatment and dilution. All plates were incubated in an anaerobic environment at 37°C for 72 hours, at which point the numbers of colony forming units (cfu) were determined.

STATISTICAL ANALYSIS

Confidence intervals - Confidence intervals were calculated at 95% to give a range of values, consistent with the data, that is believed to encompass the actual or "true" population value.

P-values - P-values were calculated to measure the significance of the statistical evidence in favor of the null hypotheses:

1. Active and inactive Sonicare Elite remove the same percentage of bacteria.
2. Active and inactive Braun Oral-B 3D remove the same percentage of bacteria.
3. Sonicare Elite and Braun Oral-B 3D removc the same percentage of bacteria.

P-values below 0.05 can be considered to show the null hypothesis to be incorrect. The smallcr the P-value, the greater the significance of thc result.

Results

The separation of the HA discs from the tips of the bristles was established by observing the lipstick removal pattern of both brushes. The distance betwcen the bristles and the nearest edge of the HA disc was 1.6 mm for both brushes. This ensured that the biofilm removal was due to fluid forces alone and not bristle contact throughout the complete brushing cycle.

The percentage of plaque biofilm removed from the interproximal model by the active Sonicare Elite was 32.23% (23.02 to 45.01% with 95% confidence; n = 60) (Fig. 2), whereas the active Braun Oral-B 3D removed 9.48% (7.72 to 11.57% with 95% confidence; n = 60). The inactive Sonicare Elite removed 0.88% (0.53 to 1.36% with 95% confidence; n = 46-48) and the inactive Braun Oral-B 3D removed 1.10% (0.96 to 1.27 with 95% confidence; n =57-60). The increase in the efficacy of interproximal plaque removal, with the powered brushes activated, was significant (*i.e.* P< 0.05) compared to the inactive brushes (Braun, P= 0.002, Sonicare, P= 0.005). The percentage of plaque removed by the Sonicare Elite beyond the bristles after 5 seconds of exposure was significantly greater than that removed by the Braun Oral-B 3D (P= 0.012).

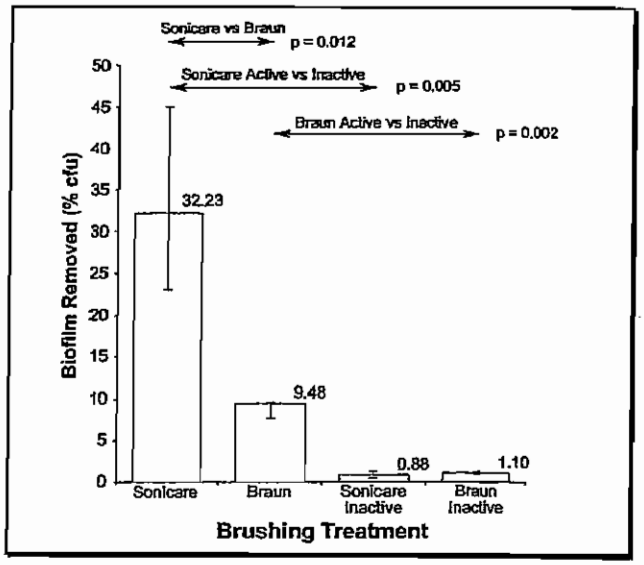

Fig. 2. Percentage of biofilm removed by Sonicare Elite and Braun Oral-B 3D.

The efficacy of plaque removal by the Sonicare Elite was 3.40 times greater (1.99 to 5.83 with 95% confidence; n = 60) than the Braun Oral-B 3D based on the percentage of bacteria removed.

Discussion

Toothbrushes, whether manually activated or electrically powered, typically rely on direct physical contact of the bristles with the tooth surface to remove dental plaque and debris from the dentition. As such, plaque removal would conceivably be limited to the region that bristles contact the tooth surface. The extent of toothbrush bristle contact upon simulated dental surfaces has been investigated using methodologies ranging from pressure sensitive indicators[21] to a robotic system using a brush to remove an artificial plaque.[22] Although these studies and evaluation systems have focused on the interproximal region, they do not have the capability to simulate a realistic representation of interproximal plaque removal; rather they primarily assess bristle contact. Soft cheese has been used to simulate "food-debris" in an assessment of the fluid dynamic capabilities of various powered brushes;[5] however, that model was not appropriate for the interproximal space. Our system is a marked improvement over previously published methods in that it simulates interproximal dental plaque using both a biofilm known to be similar to dental plaque[11] and an accurate geometrical configuration of teeth and fluid in the oral cavity.

An *in vitro* brushing system is particularly advantageous when used to ascertain objective plaque removal efficacy of different electric toothbrushes at specific locations within the oral cavity. The *in vitro* system has the advantage of eliminating many problematic factors associated with *in vivo* clinical trials. Major problems with *in vivo* studies of plaque removal include the poor reproducibility of results and the inability to standardize treatment. Natural variations in human dental plaque, both between subjects and day-to-day within a subject, also make brush comparisons challenging. A patient's personal preference for a particular brush may compromise objectivity.[23] Furthermore, *in vivo* studies are often conducted at academic research institutions where the 'dental IQ' of patients is relatively high.[24]

The results of this study indicated that power brushes do have the ability to cause the disruption of dental plaque biofilm due to the hydrodynamic fluid forces that they create. Based on these results and observations of the interproximal fluid motion in the experimental model, it appeared that various factors might have contributed to biofilm disruption. One apparent difference between brushes is the direction of fluid activation, which may be due to differences in bristle motion. During brushing experiments, the Sonicare Elite could be seen to produce an energetic stream of fluid and bubbles moving through the interproximal space, whereas the fluid activity produced by the Braun Oral-B 3D seemed to be confined principally to the same side of the dentition as the bristles.

Another factor that may contribute to biofilm disruption is the velocity of fluid flowing through the interproximal space. Although not quantified in this study, the Sonicare Elite appears to generate fluid flow with a greater velocity through the interproximal space. This fluid velocity is generated by the moving bristles or brush head and is likely related to their velocity as well as the direction of activation. Our estimates of bristle tip velocity based on the amplitude and frequency of bristle motion are 4.08 m/s for the Sonicare Elite (peak amplitude = 2.5 mm, frequency = 261 Hz) and 1.12 m/s for the Braun Oral-B (vector sum, peak amplitudes = 2.8 mm and 0.15 mm, and frequencies = 63 Hz and 170 Hz, respectively for the oscillating rotation and pulsation of the bristles[25]). The relative ratio of velocity between brushes (Sonicare Elite/ Braun Oral-B 3D) is 3.64. This value is, interestingly, similar to the ratio of biofilm removal between brushes (3.40). The force generated by a moving fluid, and likely the force upon the biofilm bacteria, is related to the velocity of the moving fluid.

Still another factor that may contribute to biofilm removal is the amount of bubbles generated by the brush. Bubbles in a fluid flow are known to influence biofilm detachment[6] presumably as they generate localized forces upon the biofilm. As mentioned previously, Sonicare Elite appeared to generate more bubble activity interproximally than the Braun Oral-B 3D. Although it is evident that a difference in ability to remove interproximal biofilm does exist between powered brushes, the contribution of various factors needs further exploration.

In this study, impartiality was afforded by independently confirming the brushing time of 5 seconds, selection of biofilms and the load between the brushes and the teeth for each experiment. The operation of the brushing machine and tooth/biofilm positions were informally validated by qualified colleagues as being both suitable models without any apparent bias towards a particular brush.

As with any study, it is only appropriate to draw comparative conclusions from results generated within the given study. Comparing the results presented here with other studies in which biofilm disruption may have been observed is inappropriate as the experimental conditions (dental model, biofilm, device, etc.) are different.

In conclusion, when the brushes were activated, both the Sonicare Elite and the Braun Oral-B 3D removed a significantly higher percentage of interproximal plaque than when their brush motions were inactivated ($P < 0.05$). This shows that the fluid forces generated by both Sonicare Elite and Braun Oral-B 3D penetrate beyond the bristles. However, the Sonicare Elite removes significantly more plaque beyond the bristles than the Braun Oral-B 3D ($P = 0.012$).

a. Philips Oral Healthcare, Inc., Snoqualmie, WA, USA.
b. Braun GmbH, Kronberg, Germany.
c. University of Wales, Cardiff, UK.
d. Clarkson Chromatography Products, South Williamsport, PA, USA.
e. Whatman, Maidstone, UK.
f. BDH Chemicals, Poole, UK.
g. Medical Air Technology Ltd., Manchester, UK.
h. Columbia Dentoform, Long Island City, NY, USA.
i. Becton Dickinson, Sparks, MD, USA.

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Exhibit M

Bacteria

I. INTRODUCTION

Bacteria, one-celled organisms visible only through a microscope. Bacteria live all around us and within us. The air is filled with bacteria, and they have even entered outer space in spacecraft. Bacteria live in the deepest parts of the ocean and deep within Earth. They are in the soil, in our food, and on plants and animals. Even our bodies are home to many different kinds of bacteria. Our lives are closely intertwined with theirs, and the health of our planet depends very much on their activities.

Bacterial cells are so small that scientists measure them in units called micrometers (µm). One micrometer equals a millionth of a meter (0.0000001 m or about 0.000039 in), and an average bacterium is about one micrometer long. Hundreds of thousands of bacteria would fit on a rounded dot made by a pencil.

Bacteria lack a true nucleus, a feature that distinguishes them from plant and animal cells. In plants and animals the sacklike nucleus carries genetic material in the form of deoxyribonucleic acid (DNA). Bacteria also have DNA but it floats within the cell, usually in a loop or coil. A tough but resilient protective shell surrounds the bacterial cell.

Biologists classify all life forms as either prokaryotes or eukaryotes. Prokaryotes are simple, single-celled organisms like bacteria. They lack a defined nucleus of the sort found in plant and animal cells. More complex organisms, including all plants and animals, whose cells have a nucleus, belong to the group called eukaryotes. The word *prokaryote* comes from Greek words meaning "before nucleus"; *eukaryote* comes from Greek words for "true nucleus."

Bacteria inhabited Earth long before human beings or other living things appeared. The earliest bacteria that scientists have discovered, in fossil remains in rocks, probably lived about 3.5 billion years ago. These early bacteria inhabited a harsh world: It was extremely hot, with high levels of ultraviolet radiation from the sun and with no oxygen to breathe.

Descendents of the bacteria that inhabited a primitive Earth are still with us today. Most have changed and would no longer be able to survive the harshness of Earth's early environment. Yet others have not changed so much. Some bacteria today are able to grow at temperatures higher than the boiling point of water, 100°C (212°F). These bacteria live deep in the ocean or within Earth. Other bacteria cannot stand contact with oxygen gas and can live only in oxygen-free environments--in our intestines, for example, or in the ooze at the bottom of swamps, bogs, or other wetlands. Still others are resistant to radiation. Bacteria are truly remarkable in terms of their adaptations to extreme environments and their abilities to survive and thrive in parts of Earth that are inhospitable to other forms of life. Anywhere there is life, it includes bacterial life.

II. THE IMPORTANCE OF BACTERIA

Much of our experience with bacteria involves disease. Although some bacteria do cause disease, many kinds of bacteria live on or in the human body and prevent disease. Bacteria associated with the human body outnumber body cells by ten to one. In addition, bacteria play important roles in the environment and in industry.

A. Bacteria and Human Health

We have all had bacterial diseases. Bacteria cause many cases of gastroenteritis, sometimes called stomach flu. Perhaps the most common bacterial disease is tooth decay. Dental plaque, the sticky film on our teeth, consists primarily of masses of bacteria. These bacteria *ferment* (break down) the sugar we eat to produce acids, which over time can dissolve the enamel of the teeth and create *cavities* (holes) in the teeth.

Tooth decay provides a good example of how multiple factors contribute to bacterial disease. The human body hosts the bacteria, the diet supplies the sugars, and the bacteria produce the acid that damages the teeth.

A.1. Bacteria That Inhabit the Body

Communities of bacteria form what are called *biofilms* on many body surfaces. Dental plaque is a biofilm covering the teeth. Biofilms also cover the soft tissues of our mouths and the inner surfaces of our nose, sinuses, throat, stomach, and intestines. Even the skin has bacterial communities that extend into hair follicles. Bacterial communities differ in each region of the body, reflecting the environmental conditions in their specific region. Bacteria that inhabit the surface of the stomach, for example, must deal with extremely strong acid in the digestive juices.

Some regions in the interior of the body are sterile—that is, devoid of living organisms other than the cells of the body. Sterile regions include the muscles, the blood, and the nervous system. However, even these regions face constant invasion by bacteria. The body's immune system is designed to rid the body of these invaders.

A healthy, balanced community of bacteria is extremely important for our health. Some of these organisms protect us from disease-causing organisms that would otherwise infect us. Animals raised in a completely germ-free environment, without any contact with bacteria, are highly susceptible to infectious diseases if they are exposed to the outside world. Bacteria in our bodies also provide us with needed nutrients, such as vitamin K, which the body itself cannot make. The communities of bacteria and other organisms that inhabit the body are sometimes called the normal microflora or microbiota.

A.2. Disease-Causing Bacteria

In most cases the bacteria that cause disease are not part of the bacteria that normally inhabit the body. They are picked up instead from sick people, sick animals, contaminated food or water, or other external sources. Bacterial disease also can occur after surgery, an accident, or some other event that weakens the immune system.

A.2.a. Opportunistic Infections

When the immune system is not functioning properly, bacteria that usually are harmless can overwhelm the body and cause disease. These organisms are called *opportunistic* because they cause disease only when an opportunity is presented. For example, cuts or injuries to the skin and protective layers of the body enable normally friendly bacteria to enter the bloodstream or other sterile parts of the body and cause infection. Surgery may enable bacteria from one part of the body to reach another, where they cause infection. A weakened immune system may be unable to prevent the rapid multiplication of bacteria and other microorganisms.

Opportunistic infections became more important in the late 20th century because of diseases such as acquired immunodeficiency syndrome (AIDS), a viral disease that ravages the immune system. Also contributing to an increase in opportunistic infections is the wider use of cancer-fighting drugs and other drugs that damage the immune system.

A.2.b. Bacterial Killers

Some dramatic infectious diseases result from exposure to bacteria that are not part of our normal bacterial community. Cholera, one of the world's deadliest diseases today, is caused by the bacterium

Vibrio cholerae. Cholera is spread in water and food contaminated with the bacteria, and by people who have the disease. After entering the body, the cholera bacteria grow in the intestines, often along the surface of the intestinal wall, where they secrete a *toxin* (poison). This toxin causes massive loss of fluid from the gut, and an infected person can die of *dehydration* (fluid loss) unless the lost fluids, and the salts they contain, are replaced. Cholera is common in developing regions of the world that lack adequate medical care.

Another major bacterial killer is *Mycobacterium tuberculosis*, which causes tuberculosis (TB), a disease of the lungs. Tuberculosis is responsible for more than 2 million deaths per year worldwide. Although antibiotics such as penicillin fight many bacterial diseases, the TB bacterium is highly resistant to most antibiotics. In addition, the TB-causing bacteria readily spread from person to person.

A.2.c. New Bacterial Diseases

While tuberculosis and cholera have been with us for centuries, in recent decades new bacterial diseases have emerged. Legionnaires' disease, a severe form of pneumonia, was first recognized at an American Legion convention in Philadelphia, Pennsylvania, in 1976. It is caused by a previously unknown bacterium, *Legionella pneumophila*, which is most often transmitted through infected water.

Lyme disease, a form of arthritis caused by the bacterium *Borrelia burgdorferi*, was first recognized in Lyme, Connecticut, in 1975. A bite from a deer tick that carries the bacteria transmits the disease to human beings.

A food-borne disease currently causing major concern in the United States, Canada, and Western Europe is caused by a particular variant of the common intestinal bacterium *Escherichia coli*, or *E. coli* for short. Although *E. coli* is normally present in the human intestines, the variant *E. coli O157:H7* produces toxins that cause bloody diarrhea and, in some cases, far more severe problems, including kidney failure and death. A person can become infected by eating contaminated meat. Thorough cooking kills the bacteria.

A.3. How the Body Fights Bacterial Disease

Our immune system is designed to protect us against harmful bacteria. It works to keep our normal microflora in check and also to eliminate invaders from outside the body. Some immune-system defenses are built in: The skin acts as a barrier to bacterial invaders, and antimicrobial substances in body secretions such as saliva and mucus can kill or stop the growth of some disease-causing bacteria. We acquire another immune-system defense through exposure to disease-causing bacteria.

After recovering from many bacterial infections, people have the ability to resist a second attack by the same bacteria. They can do so because their immune system forms disease-fighting proteins called antibodies designed to recognize specific bacteria. When next exposed to those bacteria, the antibodies bind to the surface of the bacteria and either kill them, prevent them from multiplying, or neutralize their toxin. Vaccines also can stimulate the immune system to form disease-fighting antibodies. Some vaccines contain strains of the bacterium that lack the ability to cause infection; others contain only parts of bacterial cells.

A.4. Treatment and Prevention of Bacterial Disease

A.4.a. Antibiotics

In many cases the immune system can wipe out a bacterial infection on its own. But sometimes people become so sick from a bacterial disease that they require medical treatment. Antibiotics and other antibacterial drugs are the major weapons against disease-causing bacteria. Antibiotics act in a number of ways to kill bacteria or suppress their activity. Over time, however, bacteria can become resistant to antibiotics. As a result bacterial diseases have become more and more difficult to cure.

In an effort to control antibiotic resistance, physicians have tried to limit the use of antibiotics. In addition, they have advocated more vigorous efforts to improve the antibiotics we now have and to find new agents active against bacteria.

A.4.b. Vaccines

Immunization through vaccines is important in the prevention of infectious diseases caused by bacteria. Vaccines expose a human being or other animal to a disease-causing bacterium or its toxins without causing the disease. As a result of this exposure, the body forms antibodies to the specific bacterium. These antibodies remain ready to attack if they meet the bacteria in the future. Some immunizations last a lifetime, whereas others must be renewed with a booster shot.

Tetanus provides a good example of a successful vaccine. The bacterium *Clostridium tetani,* found in soil and ordinary dirt, produces one of the most lethal toxins known. The toxin affects nerves, resulting in muscle rigidity and death. Tetanus infection has become very rare in developed countries such as the United States where nearly everyone is immunized against the toxin. The vaccine immunizes the body by means of toxins that have been chemically treated so they are no longer toxic. Health officials recommend getting a tetanus shot every ten years. In less developed countries where vaccination is not so common, tetanus is a major cause of death, especially of babies.

A.4.c. Public Health Measures

Public health measures provide major controls against infectious disease. Especially important are those measures leading to ready availability of clean water, safe food, and up-to-date medical care. Waterborne diseases, such as cholera and typhoid fever, kill an estimated 5 million to 10 million people worldwide each year, according to the United Nations. Sufficient sources of clean drinking water in developing countries could help prevent these deaths. Food-safety guidelines can help prevent the spread of disease through contaminated food. Proper medical care can prevent transmission of infectious diseases to others. Tuberculosis, for example, kills more people worldwide every year than any other single disease. But if identified early, cases of tuberculosis can be treated effectively with antibiotics and other means, thereby stopping transmission to others.

Maintaining a clean environment for medical care is also important in preventing the spread of infectious diseases. For example, medical instruments, such as needles and syringes, must be sterile and proper infection-control procedures must be followed in hospitals, medical and dental offices, and industries that use bacteria. However, it is never possible, or even desirable, to have an environment entirely free of bacteria.

B. Bacteria and the Environment

Bacteria play a major role in recycling many chemical elements and chemical compounds in nature. Without such bacterial activities as the recycling of carbon dioxide (CO_2) life on Earth would be impossible. Plants use CO_2 to grow and in the process they produce the oxygen humans and other animals breathe. Moreover, we would drown in garbage and wastes if bacteria did not speed the decomposition of dead plant and animal matter.

B.1. Nitrogen Fixation

Bacteria play a key role in making soil fertile. They convert nitrogen in Earth's atmosphere into the nitrogen compound ammonia, which plants need to grow. Bacteria are the only organisms able to carry out this biochemical process known as nitrogen fixation. The bacteria able to fix atmospheric nitrogen usually live in association with plants, often integrated into the plant tissue. Bacteria in the genus *Rhizobium,* for example, form *nodules* (knobs) on the roots of beans and other plants in the legume family.

B.2. The Carbon Cycle

Bacteria and *fungi* (yeasts and molds) are vital to another process that makes life on Earth possible: the carbon cycle. They help produce the gas carbon dioxide (CO_2), which plants take from the atmosphere. During a part of the carbon cycle called photosynthesis, plants turn sunlight and CO_2 into food and energy, releasing oxygen into the atmosphere.

The carbon cycle continues after plants and animals die, when bacteria help convert the material of which those organisms are made back into CO_2. Bacteria and fungi secrete enzymes that partially break down dead matter. Final digestion of this matter takes place within bacterial and fungal cells by the processes of fermentation and respiration. The CO_2 released by this action escapes back into the atmosphere to renew the cycle.

B.3. Chemosynthesis

Bacteria are major players in cycles of other elements in the environment. Chemosynthetic bacteria use chemical energy, instead of the light energy used by plants, to change CO_2 into something that other organisms can eat. Chemosynthesis occurs in vents at the bottom of the ocean, where light is unavailable for photosynthesis but hydrogen sulfide gas, H_2S, bubbles up from below Earth's crust. Life can develop around these vents because bacteria use the H_2S in changing CO_2 into organic nutrients. The H_2S coming up from Earth's mantle is extremely hot, but bacteria in these vent communities are adapted to the high temperatures. Bacteria's ability to react chemically with sulfur compounds is useful in certain industrial processes as well.

B.4. Bioremediation

Bioremediation refers to the use of microorganisms, especially bacteria, to return the elements in toxic chemicals to their natural cycles in nature. It may provide an inexpensive and effective method of environmental cleanup, which is one of the major challenges facing human society today.

Bioremediation has helped in cleaning up oil spills, pesticides, and other toxic materials. For example, accidents involving huge oil tankers regularly result in large spills that pollute coastlines and harm wildlife. Bacteria and other microorganisms can convert the toxic materials in crude oil to harmless products such as CO_2. Adding fertilizers that contain nitrogen, phosphorus, and oxygen to the polluted areas promotes the multiplication of bacteria already present in the environment and speeds the cleanup process.

C. Bacteria in Agriculture and Industry

Many of bacteria's beneficial roles in agriculture have been described in the previous section on Bacteria and the Environment. By recycling certain chemical elements and compounds, bacteria make plant and animal life possible. Bacteria's chemical interactions have also found uses in industry. In recent decades, scientists have engineered bacterial genes to produce sought-after substances, such as human insulin, to use in the treatment of disease.

C.1. Bacteria in Agriculture

Through the process of nitrogen fixation, bacteria turn nitrogen in the air into nutrients that crops and other plants need to grow. Some of the nitrogen-fixing bacteria attach to the roots of plants. Through the carbon cycle, bacteria produce the carbon dioxide that plants require for photosynthesis. Bacteria that live in the stomachs of cud-chewing animals, such as cows and sheep, help the animals digest grasses.

Bacteria also can be harmful in agriculture because of the major diseases of farm animals they cause. Many of the bacteria that cause infectious diseases in farm animals resemble those that cause similar

human diseases. For example, a variant of the bacterium that causes human tuberculosis causes tuberculosis in cattle, and it can infect humans through cow's milk. To prevent transmission of the disease, milk for human consumption should be *pasteurized* (heated at a temperature between 60° and 70°C (140° and 158°F) for a short time. Pasteurization kills most bacteria in milk.

Other disease-causing bacteria primarily affect animals other than humans. For example, the bacterium *Brachyspira hyodysenteria* causes a type of diarrhea in pigs that can be disastrous for pig farmers. Many infectious diseases of farm animals also affect wild animals, such as deer. Wild animals, in turn, can infect domestic animals, including cats and dogs.

C.2. Bacteria in the Food Industry

Bacteria are of major importance in the food industry. On the one hand, they cause food spoilage and foodborne diseases, and so must be controlled. On the other hand, they improve food flavor and nutrition.

The dairy industry provides prime examples of bacteria's harmful and helpful roles. Before the introduction of pasteurization in the late 1800s, dairy products were major carriers for bacteria that caused such illnesses as tuberculosis and rheumatic heart disease. Since that time regulation of the dairy industry has greatly reduced the risks of infection from dairy products.

On the helpful side, bacteria contribute to the *fermentation* (chemical breakdown) of many dairy products people eat every day. Yogurt, considered a healthful food, is produced by bacterial fermentation of milk. The bacteria produce lactic acid, which turns the milk sour, hampers the growth of disease-causing bacteria, and gives a desirable flavor to the resulting yogurt. Cheese also is produced by fermentation. First, bacteria ferment milk sugar to lactic acid. Then, cheese makers can introduce various microorganisms to produce the flavors they desire. The process is complicated and may take months or even years to complete, but it gives cheeses their characteristic flavors.

The variety of fermented foods we eat ranges from pickles, olives, and sauerkraut to sausages and other cured meats and fish, chocolate, soy sauce, and other products. In most of these fermentations, bacteria that produce lactic acid play major roles. Alcohol-producing yeasts are the primary fermentors in the manufacture of beer and wine, but lactic-acid bacteria also are involved, especially in making wine or cider. Bacteria that produce acetic acid can convert wine, cider, or other alcoholic beverages to vinegar.

C.3. Bacteria in Waste Treatment

Bacteria are very important in sewage treatment. Standard sewage treatment involves multiple processes. It usually starts with settling during which large items sink to the bottom. Next, air is bubbled through the sewage. This so-called aerobic phase encourages oxygen-using bacteria to break down organic material in the sewage, such as human wastes, to acids and CO_2. Most disease-causing organisms are also killed in this phase. The sewage sludge left behind is attacked in a subsequent phase by *anaerobic bacteria* (bacteria that cannot tolerate oxygen). These bacteria break down the sludge to produce methane gas, which can then be used as a fuel to power the treatment facility. In treatment plants today, this anaerobic phase sometimes precedes the aerobic phase.

Bacteria are also effective in cleaning up harmful wastes through bioremediation. In this process bacteria and other microorganisms convert toxic or otherwise objectionable wastes, such as pesticides and oil spills, to harmless or even useful products.

C.4. Bacteria in Mineral Extraction

An interesting industrial process carried out by bacteria is the recovery of valuable minerals such as copper from ores. The most important copper ores are copper sulfides, which may contain only a small percentage of copper. Bacteria of the genera *Thiobacillus* and *Sulfolobus* are able to oxidize sulfides—that is, cause a chemical reaction of sulfides with oxygen—yielding sulfuric acid. This action produces the acid conditions necessary to *leach* (remove) the copper from the ores. The use of

bacteria in extracting minerals, though slow, is environmentally friendly compared with the standard process of smelting. Smelting requires energy to heat the ore to extremely high temperatures for extracting minerals, and it also releases gases that pollute the air.

Some chemical reactions in which bacteria participate are harmful rather than helpful to industry. Bacteria are major agents of metal *corrosion* (wearing away) through the formation of rust, especially on metals containing iron. During the early stages of rust formation, hydrogen is produced, and it acts to slow the rusting process. However, certain bacteria use the hydrogen as a nutrient with the result that they greatly speed up rust formation.

C.5. Bacteria in Biotechnology

Bacteria have been at the center of recent advances in biotechnology—the creation of products for human benefit through the manipulation of biological organisms. Biotechnology itself dates back at least as far as ancient Egyptian civilization. Paintings on the walls of Egyptian tombs depict the brewing of beer, which uses microorganisms in the fermentation process. However, the existence of bacteria did not become known until the development of sufficiently powerful microscopes in the late 1600s. During the centuries that followed, scientists became aware that living organisms were responsible for many biotechnological processes.

Biotechnology grew steadily during the 20th century. In the 1970s scientists used information about replication of viruses and bacteria and about DNA *synthesis* (manufacture) to begin the genetic engineering of bacterial cells. When scientists combined human DNA with the DNA in bacterial cells, recombinant DNA technology was born. Human DNA is the "recombinant." DNA contains the instructions for creating proteins. With their recombinant DNA, bacteria became factories for turning out human proteins, such as the hormone insulin or antibodies that fight disease. Because they multiply so rapidly, bacteria produce multiple copies of proteins in a short time. The process of taking genetic information from one organism and placing it in a different organism was patented by American biochemists Stanley Cohen and Herbert Boyer in 1980. The genetic revolution was underway.

C.6. Other Industrial Roles

Bacteria play a role in the production of other products, including certain plastics and enzymes used in laundry detergents. They also produce many antibiotics, such as streptomycin and tetracycline. Since the 1980s, bacteria have gained importance in the production of many bulk chemicals, including ethanol, a form of alcohol made from fermented corn. Ethanol is an ingredient of gasohol, a fuel that burns more cleanly than gasoline and uses less petroleum. Chemical production using bacteria and other microorganisms results in less pollution to the environment than standard chemical production. The growth of genetic engineering has opened the way to even greater use of bacteria in large-scale industrial manufacturing and environmentally friendly processes.

C.7. Controlling Bacterial Growth

Sterilization and disinfection—processes for destroying microorganisms—are integral parts of the food industry. For example, canning involves heating foods to temperatures of 121°C (250°F) to kill all organisms, including the most heat-resistant bacterial cells. Failure to kill bacteria and the spores they produce can result in fatal disease such as botulism. If spores of the bacterium *Clostridium botulinum* are not destroyed, they can grow in canned foods and produce a toxin that attacks the nervous system. The botulism toxin is one of the most deadly toxins known.

Demand for better sterilization and disinfection methods in medicine and other industries has increased since the 1970s because of fear of spreading infection by the human immunodeficiency virus (HIV) and other disease-causing microorganisms. Industry has developed a wide array of products oriented to killing bacteria and other organisms. The industry has grown to be a huge one with a wide array of products oriented to killing bacteria and other microorganisms.

III. CHARACTERISTICS OF BACTERIA

Bacteria are so small that they can be seen only under a microscope that magnifies them at least 500 times their actual size. Some become visible only at magnifications of 1,000 times. They are measured in micrometers (µm) and average about 1 to 2 µm in length. One micrometer equals one-millionth of a meter (0.0000001 m or about 0.000039 in).

Bacteria not only have many uses, they also occur in diverse shapes and types. As a group they carry out a broad range of activities and have different nutritional needs. They thrive in a variety of environments.

A. Types of Bacteria

Scientists use various systems for classifying bacteria into different types. One of the simplest systems is by shape. Other systems depend on oxygen use, source of carbon, and response to a particular dye.

A.1. Classification by shape

Most bacteria come in one of three shapes: rod, sphere, or spiral. Rod-shaped bacteria are called *bacilli*. Spherical bacteria are called *cocci*, and spiral or corkscrew-shaped bacteria are called *spirilla*. Some bacteria come in more complex shapes. A hairlike form of spiral bacteria is called *spirochete* (*see* Spirochetes). Streptococci and staphylococci are well-known disease-causing bacteria among the cocci.

A.2. Aerobic and Anaerobic Bacteria

Scientists also classify bacteria according to whether they need oxygen to survive or not. Aerobic bacteria require oxygen. Anaerobic bacteria cannot tolerate oxygen. Bacteria that live in deep ocean vents or within Earth are anaerobic. So are many of the bacteria that cause food poisoning.

A.3. Autotrophic and Heterotrophic Bacteria

All bacteria require carbon for growth and reproduction. Bacteria called autotrophs ("self-feeders") get their carbon from CO_2. Most bacteria, however, are heterotrophs ("other feeders") and derive carbon from organic nutrients such as sugar. Some heterotrophic bacteria survive as parasites, growing within another living cell and using the nutrients and cell machinery of their host cells. Some autotrophic bacteria, such as cyanobacteria, use sunlight to produce sugars from CO_2. Others depend instead on energy from the breakdown of inorganic chemical compounds, such as nitrates and forms of sulfur.

A.4. Gram-Positive and Gram-Negative Bacteria

Another system of classifying bacteria makes use of differences in the composition of cell walls. The difference becomes clear by means of a technique called Gram's stain, which identifies bacteria as either gram-positive or gram-negative. After staining, gram-positive bacteria hold the dye and appear purple, while gram-negative bacteria release the dye and appear red. Gram-positive bacteria have thicker cell walls than gram-negative bacteria. Knowing whether a disease-causing bacterium is gram-positive or gram-negative helps a physician to prescribe the appropriate antibiotic. The stain is named for H. C. J. Gram, a Danish physician who invented it in 1884.

A.5. The Cell and Its Structure

The cell wall generally determines the shape of the bacterial cell. The wall is a tough but resilient shell that keeps bacterial cells from drying out and helps them resist environmental stress. In some cases the cell wall protects the bacterium from attack by the body's disease-fighting immune system cells. Some bacteria do not have much of a cell wall, while others have quite thick structures. Many species of bacteria move about by means of flagella, hairlike structures that project through the cell wall. The

flagellum's rotating motion propels the bacterial cell toward nutrients and away from harmful substances.

Like all cells bacteria contain the genetic material DNA. But bacterial DNA is not contained within a nucleus, as is DNA in plant and animal cells. Most bacteria have a single coil of DNA, although some bacteria have multiple pieces. Bacterial cells often have extra pieces of DNA called plasmids, which the cell may gain or lose without dying. Surrounding the DNA in a bacterial cell is cytoplasm, a watery fluid that is rich in proteins and other chemicals. A cell membrane inside the wall holds together the DNA and the constituents of the cytoplasm. Most activities of the bacterial cell are carried out within the cytoplasm, including nutrition, reproduction, and the manufacture of proteins.

B. How Bacteria Function

Bacterial cells, like all cells, require nutrients to carry out their work. These nutrients must be water soluble to enter through pores in the cell wall and pass through the cell membrane into the cytoplasm. Many bacteria, however, can digest solid food by secreting chemicals called exoenzymes into the surrounding environment. The exoenzymes help break down the solid food outside the bacteria into water-soluble pieces that the cell wall can absorb. Bacterial cells use nutrients for a variety of life-sustaining biochemical activities known collectively as metabolism.

B.1. Anabolism and Catabolism

The metabolic activities that enable the cell to function occur in two ways: anabolism and catabolism. Simply put, anabolism is the manufacture of complex molecules from simple ones, and catabolism is the breakdown of complex molecules into simple ones. Cells use the energy from catabolism for all their other tasks, including growth, repair, and reproduction.

A single bacterial cell takes up small molecules from the environment by means of specific transport proteins in the cell membrane. In the case of more complex molecules, such as proteins or complex carbohydrates, bacteria first secrete digestive enzymes into the environment to break the nutrients down into smaller molecules, which are transported across the membrane. *Enzymes* (proteins that speed chemical reactions) within the cytoplasm then digest the molecules further. This breakdown, called catabolism, results in energy transfer through the processes of respiration and fermentation. During metabolism, some of the small molecules are converted into the molecules the cell needs to *synthesize* (manufacture) its own proteins, nucleic acids (building blocks of DNA), lipids (fatty substances), and polysaccharides (sugars and starches). The metabolic processes for synthesis of these complex cells are anabolism.

B.2. Adaptation to Environmental Stress

All organisms have some capacity to adapt to environmental stress, but the extent of this adaptive capacity varies widely. Heat, cold, high pressure, and acid or alkaline conditions can all produce stress. Bacteria easily adapt to environmental stress, usually through changes in the enzymes and other proteins they produce. These adaptations enable bacteria to grow in a variety of conditions. Gradual exposure to the stress, for example, may enable bacteria to synthesize new enzymes that allow them to continue functioning under the stressing conditions or that enhance their capacity to deal with the stressing agent. Or they may resist environmental stress in other ways. Some bacteria that live in extremely acidic conditions can pump out acid from their cell.

Extremophiles are organisms that can grow in conditions considered harsh by humans. Some kinds of bacteria thrive in hydrothermal vents on the ocean floor or in oil reservoirs within Earth, at high pressures and temperatures as high as 120°C (250°F). Other kinds can live at temperatures as low as −12°C (10°F) in Antarctic brine pools. Other bacteria have adapted to grow in extremely acid conditions, where mines drain or minerals are leached from ores and sulfuric acid is produced. Others grow at extremely alkaline or extremely salty conditions. Still others can grow in the total absence of oxygen. Bacteria able to function in these extreme conditions generally cannot function under conditions we consider normal.

B.3. Reproduction and Survival

Bacteria reproduce very rapidly. Replication in some kinds of bacteria takes only about 15 minutes under optimal conditions. One bacterial cell can become two in 15 minutes, four in 30 minutes, eight in 45 minutes, and so on. Bacteria would quickly cover the entire face of the globe if their supply of nutrients was unlimited. Fortunately for us, competition for nutrients limits their spread. In the absence of sufficient nutrients, however, many bacteria form dormant spores that survive until nutrients become available again. Spore formation also enables these bacteria to survive other harsh conditions.

B.3.a. Binary Fission

The simplest sort of bacterial reproduction is by *binary fission* (splitting in two). The bacterial cell first grows to about twice its initial size. Toward the end of that growth, the cell membrane forms a new membrane that extends inward toward the center of the cell. The cell wall follows closely behind, bisecting the cell. The membrane then seals to divide the enlarged cell into two small cells of equal or nearly equal size, and a new wall forms between the membranes.

The growth and division of a bacterial cell has two main phases. In one phase, the cell replicates its DNA and makes all the other molecules needed for the new cell. The second phase—cell division—occurs when DNA replication stops. In the bacterium *E. coli* replication takes about 40 minutes and cell division lasts about 20 minutes. The entire cycle takes about an hour. Yet the time for one cell to become two cells still takes only about 20 minutes. How is this possible? The cell does not wait for one cycle of replication to end before it starts another. Thus, a rapidly growing bacterial cell is carrying out multiple rounds of replication at the same time.

B.3.b. Spore Formation

In response to limited nutrients or other harsh conditions, many bacteria survive by forming spores that resist the environmental stress. Spores preserve the bacterial DNA and remain alive but inactive. When conditions improve, the spore *germinates* (starts growing) and the bacterium becomes active again.

The best-studied spores form within the bodies of Bacillus and Clostridium bacteria, and are known as endospores. *Clostridium botulinum* spores cause deadly botulism poisoning. Endospores have thick coverings and can resist environmental stress, especially heat. Even boiling in water does not readily kill them. But they can be killed by heating in a steel vessel filled with steam at high temperature and high pressure. Endospores can live for centuries in their dormant state.

Some bacteria form other types of spores. These spores are usually dormant but not as heat resistant or long-lived as endospores. Some aquatic bacteria, for example, attach to surfaces and produce swarmer cells during division. The swarmer cell swims away to attach to another surface and give rise to still more swarmer cells. Still other bacteria survive by forming colonies made up of millions of cells that act in a coordinated way to keep the organism alive.

B.3.c. Genetic Exchange

Bacterial cells often can survive by exchanging DNA with other organisms and acquiring new capacities, such as resistance to an antibiotic intended to kill them. The simplest method of DNA exchange is genetic transformation, a process by which bacterial cells take up foreign DNA from the environment and incorporate it into their own DNA. The DNA in the environment may come from dead cells. The more the DNA resembles the cell's own DNA, the more readily it is incorporated.

Another means of genetic exchange is through incorporation of the DNA into a virus. When the virus infects a bacterial cell, it picks up part of the bacterial DNA. If the virus infects another cell, it carries with it DNA from the first organism. This method of DNA exchange is called transduction.

Transformation and transduction generally transfer only small amounts of DNA, although bacterial geneticists have worked to increase these amounts. Many bacteria are also capable of transferring large amounts of DNA, even the entire *genome* (set of genes), through physical contact. The donor cell generally makes a copy of the DNA during the transfer process so it is not killed. This method of exchange is called conjugation. DNA exchange enables bacteria that have developed antibiotic-resistant genes to rapidly spread their resistance to other bacteria.

IV. CLASSIFICATION AND STUDY OF BACTERIA

Scientists long had difficulty classifying bacteria in relation to each other and in relation to other living things. Because bacteria are so small, scientists found it nearly impossible to identify characteristic structures on or in the organisms that would help in classification. For many years bacteria were considered to be plants and named according to the botanical system of classification, by genus and species. For example, *Escherichia coli* belongs to the genus *Escherichia* and to the species *coli* within that genus. The genus name starts with a capital letter; the species name, with a small letter. Both are written in italic letters. For convenience, people often use only the letter of the genus name, as in *E. coli*, for example.

A. A New Classification System

The development of the field of molecular phylogeny in the 1970s changed our view of bacteria. Phylogeny relates organisms through their evolutionary origins. In molecular phylogeny, scientists look for similarities in the molecules of organisms to figure out relationships. Initially, scientists looked at proteins, which are made up of long strings of amino acids. They figured that if a particular protein in two organisms contained exactly the same amino acids in the same order, then the two were very closely related or even identical. If there were only a few differences, the organisms were closely related. The more differences there were, the more distant the relationship would be.

Carl Woese, a microbiologist at the University of Illinois, discovered that it was easier to work with nucleic acids, such as DNA and RNA. He found that the best molecules were ribonucleic acid molecules from ribosomes (rRNA). Ribosomes are the biochemical machines inside cells that coordinate the synthesis of proteins. It was relatively easy to obtain rRNA, to identify its chemical building blocks known as nucleotides, and to determine the order of the nucleotides in the molecule. Because rRNA shows relatively little variation from one generation to the next, it proved to be an excellent tool for determining evolutionary relationships.

Molecular phylogeny indicated that there are three major groups, or kingdoms, of organisms. One kingdom, called Eukaryotae, consists of all organisms with a true nucleus and includes all plants and animals. The two other kingdoms, called Archaea and Eubacteria, consist of prokaryotic bacteria without a true nucleus. Archaea, or archaeabacteria, were once classified with other bacteria and the two kingdoms share many characteristics. Many of the archaea are extremophiles and can live in extremely hot, salty, or acid environments, but so can many eubacteria.

The classification of bacteria into two kingdoms, a system proposed by Woese, is based almost entirely on the structure of ribosomal RNA. But it appears to agree with other findings regarding the basic structures of the organisms, their metabolism, and their evolution.

B. Sequencing Bacterial DNA

Amazing advances in technology have enabled scientists to sequence the entire genome of many bacteria—that is, identify the nucleotides that make up the DNA and the order in which the nucleotides are arranged. This knowledge, and the sciences that have developed around it, will enable scientists to harness the useful capabilities of bacteria in agriculture, industry, and other fields and to develop new drugs. In one example, scientists have turned bacteria into factories for producing the hormone insulin by inserting human insulin-producing genes into bacteria. The insulin produced can be used to treat human diabetes.

Insulin is a protein, and genes govern the production of proteins by a cell. The study of protein

production will help scientists understand the process of disease at a cellular level and help them develop new means of combating diseases. As scientists study how bacteria attach to and enter healthy cells, cause illness, and spread, they are learning useful details about the molecular structure of cells.

V. EVOLUTION OF BACTERIA

The oldest fossils of bacteria-like organisms date back as many as 3.5 billion years, making them the oldest-known fossils. These early bacteria could survive in the inhospitable conditions when Earth was young, extremely hot, and without oxygen. With the help of molecular phylogeny, scientists have pieced together a view of the evolution of bacteria. They believe that the kingdoms Archaea and Eubacteria had a common ancestor but separated very early on, a few billion years ago. Archaea may be the most common organisms on Earth today. Many of them can live without oxygen and without sunlight and inhabit such places as deep-sea vents. However, scientists currently know much more about the kingdom Eubacteria than the kingdom Archaea, because humans have more contact with disease-causing Eubacteria, such as streptococci and *E. coli,* and with Eubacteria such as lactobacilli used in food processing and other industries.

Over time, bacteria evolved to capture energy from the Sun's light and thereby carry out the process of photosynthesis, converting sunlight into nutrients. Next they developed the sort of photosynthesis that plants today carry out by splitting water molecules to produce oxygen. With oxygen available, organisms that require it, such as animals, could inhabit Earth.

Recent discoveries suggest that Eukaryotae (plants and animals) probably evolved from Eubacteria. Many of the *organelles* (structures within the cytoplasm) of plant and animal cells are actually bacterial. Among organelles derived from bacteria that invaded plant or animal cells are mitochondria and chloroplasts. Mitochondria in plants and animals convert nutrients into energy-storage molecules. Chloroplasts house the photosynthetic machinery of plant cells. Not only do bacteria live on us and in us, but we ourselves are in a way partly bacterial.

VI. SCIENTIFIC STUDY OF BACTERIA

Before the development of the microscope, some people speculated that small, invisible particles caused diseases and fermentations. But not until the late 1600s did anyone actually see bacteria. In the 1670s Dutch lens maker Antoni van Leeuwenhoek first saw what he called "wee animalcules" under his single-lens microscopes. Leeuwenhoek noticed cells of different shapes within a variety of specimens, including scrapings from his teeth and rainwater from gutters. His findings laid the foundation for the growth of microbiology.

The microscope was improved over the following centuries, but bacteria still appeared as tiny objects, even with magnifications of 1,000 times. In the 1930s, the first electron microscopes were developed. Using beams of electrons instead of light, these microscopes could magnify objects at least 200 times more than light microscopes could. With magnifications of 200,000 times actual size, it became possible to see structures within bacterial cells in detail.

Early studies of bacteria were difficult. In any environment many types of bacteria compete and cooperate, and all this activity makes it nearly impossible to figure out what each organism is doing. The first step was to separate different types of bacteria. One way of isolating bacteria was to grow them on a solid surface. Scientists first used kitchen foods, such as a potato slice cut with a sterile knife, on which to grow bacteria that attack plants. This method was not very convenient, however.

The perfect *medium* (environment) for growing bacteria also came from the kitchen, although its usefulness was demonstrated in the laboratory of German scientist Robert Koch. The medium was agar, a gel-forming substance that comes from seaweed. A coworker of Koch's noted that his wife's puddings remained solid in summer heat, whereas the gelatin on which he grew bacteria dissolved or got eaten by the bacteria. The firm puddings contained agar.

Agar dissolves in water only at temperatures close to boiling. When it cools, it forms a stable gel.

Most bacteria cannot digest it. Bacteriologists could transfer a bacterial specimen onto a plate of agar using sterile wires or loops, and obtain a colony of organisms. If more than one type of bacteria formed a colony, the scientists could repeat the process, growing each type on a separate agar plate to obtain a pure *culture* (laboratory-grown specimen) for study. They could also add nutrients to agar to provide the bacteria with the food they need for growth. In addition, they could add substances to suppress the growth of unwanted bacteria but not the growth of those the bacteriologist wished to isolate. Growing bacteria on agar has become routine in laboratories.

Bacteriologists have become accustomed to studying individual types of bacteria in pure cultures. In nature, however, bacteria usually live in diverse communities, often with hundreds of types of organisms. These communities form sticky masses called biofilms on soil particles, ocean debris, plants and animals, and just about any solid or liquid surface. In our bodies, biofilms develop on teeth, on the soft tissues of the mouth and throat, on the membrane lining the nose and sinuses, in the gut, and on all other exposed body surfaces. In nature, organisms form microbial mats on surfaces between water and air. In sewage treatment, bacteria clump together in masses. All these communities are highly diverse, harboring many kinds of organisms. They can be compared to cities in which the different members have different functions, all important to maintaining the community.

Bacteriologists are realizing more and more the need to move from studying pure cultures containing only a single species to the study of communities in biofilms and microbial mats. The growth of molecular biology and the capacity to study bacteria in molecular detail have demonstrated that the bacterial world is far more diverse than previously thought. It seems possible that we currently have discovered only a small fraction of existing types of bacteria in the world. Perhaps as many as 95 percent of total types remain unknown.

Recent information on the true diversity of bacteria comes from a study published in 2006 that used a new DNA-identification technique to study microbes taken from the ocean. Scientists found over 20,000 types of bacteria in a liter of sea water—over ten times the biodiversity predicted. Much of the diversity came from rare bacteria that had not been detected in previous studies of marine microbes. Samples were taken at eight sites in the Atlantic Ocean and Pacific Ocean from a wide range of depths and environments, including the North Sea and hydrothermal vents. The work will be expanded in the future to sample marine microbes from over 1000 ocean sites with even more types of environments. The international research is being conducted as part of the global Census of Marine Life, a ten-year project that began in 2000. The newly recognized complexity of ocean bacteria could lead to a much greater gene pool for a range of scientific work.

Scientists have already sequenced the entire genome for many bacteria. Researchers can cut pieces from bacterial DNA and replicate it in many copies. Through DNA transfer, the pieces can be inserted in bacterial cells. The cells with the new DNA may then start to make new proteins they were unable to make previously. Thus, bacteria can be genetically engineered to make a whole range of products and to develop new functions. Genetic engineering has opened up a new world of biology and a tremendous opportunity to explore bacteria and other microorganisms and to benefit humanity from the resulting knowledge.

Contributed By:
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Exhibit N

Ability of the Sonicare® Electronic Toothbrush to Generate Dynamic Fluid Activity that Removes Bacteria

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Abstract

Two laboratory studies were performed to investigate removal of bacteria adherent to model oral surfaces by a new electronic toothbrush, the Sonicare®. Sonicare produces dynamic fluid activity and mild cavitation due to the high frequency vibration of its bristles. The purpose of the study was to determine if this fluid agitation alone could dislodge bacteria from oral surfaces. Commercially pure titanium disks were ground to a finish comparable to dental implants and coated with either *Streptococcus mutans* or *Porphyromonas gingivalis* as a model for bacterial plaque on implants. Additionally, saliva-coated hydroxyapatite disks were coated with *Actinomyces naeslundii* as a model for bacterial plaque on a tooth surface. Electron and fluorescence microscopy were utilized to quantitate bacterial adherence before and after exposure to Sonicare-induced fluid forces. Bristle tip to model surface distances of 0, 1, 2, 3, and 4 mm were investigated. It was found that after 15 seconds of exposure with direct bristle contact, the reduction in adherent bacteria was nearly 100% for all 3 species, with an average 75% reduction at a distance of 2 mm. At a 4 mm distance, the reductions were 61% for *S. mutans* and 24% for *A. naeslundii*, with a minor dislodgment of *P. gingivalis*. The results indicate that the Sonicare is able to remove bacteria from these model dental surfaces due to fluid dynamic action which extends up to 4 mm beyond the reach of the bristles. (J Clin Dent 5:89–93, 1994.)

Introduction

Standard toothbrushing relies on direct contact between the toothbrush bristles and the tooth surface to remove dental plaque. The user of a manual toothbrush places the bristles against the teeth and physically moves the bristles to scrub plaque from the surfaces the bristles touch. Powered toothbrushes move the bristles across the tooth to remove plaque while relying on the user to position the toothbrush about the dentition. Other instruments such as proxibrushes, stimudents, and floss also rely on direct contact with the plaque accumulations to clean the tooth surface. As an alternative to direct bristle contact, oral irrigators are meant to remove plaque from teeth via fluid forces. Irrigators project a high velocity fluid jet that may be directed into areas where the bristles of a toothbrush cannot reach. Although both types of devices, direct contact or irrigation, possess particular strengths, most studies have found neither device to be totally effective by itself in removing plaque accumulation on all surfaces.[1,2]

A combination of direct mechanical scrubbing and penetrating fluid motion can be found in a new powered toothbrush that uses high frequency bristle motion to create dynamic fluid activity. Utilizing research originating at the University of Washington (Seattle, WA), Optiva Corp. (Bellevue, WA) developed the Sonicare® electronic toothbrush. The bristles of the Sonicare vibrate at 260 Hz, creating mild cavitation and bubble activity about the Sonicare brush head. Previous studies have shown that sonic vibrations in fluid similar to those generated by Sonicare can alter cell surface structures of the oral bacterium *Actinomyces naeslundii* (formerly *Actinomyces viscosus* T14V) as well as remove these adherent bacteria from model dental surfaces *in vitro*.[3,4] A previous clinical study of Sonicare's efficacy demonstrated its superiority over the manual toothbrush in removing supragingival plaque, particularly in hard to reach areas such as the interproximal regions and the posterior teeth.[5]

The purpose of the current study was to investigate the ability of Sonicare to remove bacteria from model dental surfaces without direct bristle contact, i.e., whether the fluid forces alone can remove adherent oral bacteria. Two independent studies were conducted at separate sites: 1) bacterial adherence to implant surfaces was modeled using titanium disks with adherent *Streptococcus mutans* or *Porphyromonas gingivalis*; and 2) bacterial adherence to the tooth surface was modeled using saliva-coated hydroxyapatite (SHA) with adherent *A. naeslundii* (Optiva Corp.).

Materials and Methods

Bacteria

S. mutans (strain Ingbritt) were grown in brain-heart infusion broth (BHI, Difco, Detroit, MI) with 2% sucrose for 48 hrs at 37°C. *P. gingivalis* (ATCC strain 33277) were routinely grown in trypticase soy broth (TSB) containing L-cysteine hydrochloride (0.5 g/l), yeast extract (5 g/l), hemin (5 μg/l) and menadione (0.2 μg/l) (PG broth) for 48–72 hrs at 37°C anaerobically

in an atmosphere of 10% H_2, 5% CO_2, and 85% N_2. *A. naeslundii* (strain T14V) were grown in TSB supplemented with 0.1% yeast extract and 0.5% glucose for 16 hr at 37°C with continuous agitation. *A. naeslundii* were prepared for adherence assays as previously described.[3]

Adherence Models

To prepare model implant surfaces, methods described by Keller *et al.* were used.[6] Titanium (Ti) disks (12.5 mm diameter, 3.5 mm thick) obtained from commercially pure Ti-based bar stock (Alfa Products, Danvers, MA) were hand ground using a 1 µm grit diamond polishing paste to produce a final surface finish comparable to commercially available implants. For disk orientation purposes during exposure and examination, two parallel lines were scribed 3 mm from opposite edges of the disk. The disks were cleaned using methylethyl ketone (5 min), rinsed with ultra-pure water (15 min), acid-passivated with 30% nitric acid (30 min) and finally rinsed with ultra-pure water (20 min).

Chambers for adherence of the bacteria on the Ti surface were prepared as described previously.[7] Tygon tubing (12.7 mm ID, 1.6 mm wall thickness, 30 mm length) was cleaned with RBS 35, 2% (Pierce Chemicals, Rockford, IL) for 5 min, rinsed 15 min with tap water, agitated 2 min in Prosil 28, 1% dilution (PCR Inc., Gainesville, FL) and rinsed 30 min with distilled water.[6] The tubing was fitted over the Ti disk and the bottom sealed with Regisil bite registration material (L.D. Caulk Div., Dentsply Intl., Milford, DE) to achieve a fluid-tight adherence chamber. The chambers were sterilized 30 min with UV light (300 µw/cm²) prior to incubation with the bacteria.

Ti disks (n = 12) were pre-colonized with *S. mutans* by growing the bacteria for 48 hrs in BHI-2% sucrose medium in the chambers described above. At the end of the incubation period, the culture medium was removed. The Ti surface with attached *S. mutans* was carefully rinsed with sterile phosphate buffered saline (PBS, 0.05M, pH 7.0) prior to exposure to Sonicare. Additionally, separate Ti disks (n = 12) were coated with *P. gingivalis* by a previously established method.[7] For these, the Ti disks were pre-coated with 0.25 ml 10% fetal calf serum at 37°C for 1 hr and the disk surface washed 3 times with PBS. A 0.5 ml aliquot of *P. gingivalis* (5 x 10^6 cells/ml in PG broth) was placed onto the serum-coated disks and incubated anaerobically for 1 hr at 37°C. After incubation, the non-attached cells and suspending medium were carefully aspirated from the chamber and the disk surfaces washed 3 times with PBS prior to exposure to the Sonicare.

For preparing *A. naeslundii*-coated SHA disks, procedures previously established were used.[3] Hydroxyapatite disks 5.2 mm diameter, 2.4 mm thick (Calcitek, Inc., Carlsbad, CA) were cleaned in an ultrasonic water bath, rinsed in KCl buffer[8] (0.05M pH 6.0) for 1 hr, and then coated with saliva overnight. Freshly-harvested *A. naeslundii* were washed 3 times in carbonate buffer (0.1 M, pH 9.6), incubated with fluorescein isothiocyanate isomer I (FITC, 0.25 mg/ml in carbonate buffer) for 2 hr, and washed 3 times in KCl buffer. The FITC-labelled *A. naeslundii* were then added onto SHA and further incubated at room temperature for 1 hr with agitation. The disks were rinsed in KCl buffer to remove non-adherent bacteria.

Exposure to the Sonicare

For Sonicare exposure, a bacteria-coated disk was rigidly mounted in a holding chamber below the Sonicare so that the peak of the tuft of bristles furthest out from the brush handle was mounted centrally over the disk, as shown in Figure 1. Ti disks were oriented so that the activated Sonicare bristles, moving at 260 Hz (520 strokes/sec), swept parallel to the scored lines on the disk. The bristle-to-surface distance was set to either 0 (contact), 1, 2, 3, or 4 mm. PBS was placed in the holding chamber so that the fluid level reached 2 mm into the tips of the shorter Sonicare bristles. The Sonicare was then activated and moved across the disk surface, ±3 mm from the central position in a direction perpendicular to bristle movement, approximately twice every 5 sec. The exposure times were between 5 and 30 sec. Control disks were not exposed to the Sonicare, but were mounted in the holding chamber for 30 sec in a manner similar to the exposed disks. All brushings were done with a fully charged Sonicare power handle.

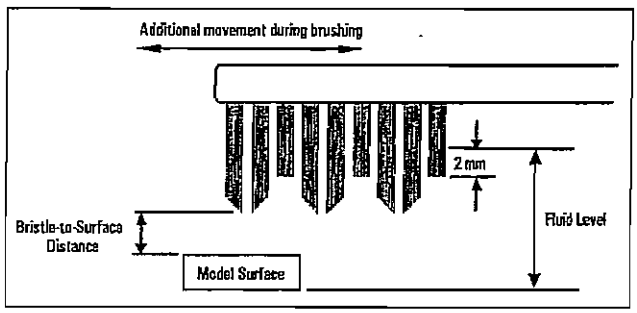

Figure 1. *The model dental surface was positioned below the Sonicare bristles and covered with fluid to a consistent level extending into the bristles. The bristle-to-surface distance was altered to investigate the ability of the Sonicare to remove bacteria from surfaces beyond the reach of the bristles. Normal Sonicare bristle movement is in and out of the page. To evenly expose the surfaces, the Sonicare was additionally moved by hand in the direction shown.*

After exposure, the disks were removed, gently rinsed with PBS or KCl buffer, and further processed for scanning electron microscopy (SEM) or fluorescent microscopy. Triplicate disks of each exposure condition for each respective bacteria were performed for the Ti study. Eight replicates of each exposure condition were performed for the SHA study. Throughout the studies, handling of the model surfaces was minimized to prevent inadvertent removal of bacteria.

Examination of the Disk Surfaces with SEM or Fluorescent Microscopy

Both control Ti disks and disks exposed to the Sonicare were rinsed twice with cacodylate buffer (CD buffer, 0.1 M, pH 7.0), placed in vials containing 3% glutaraldehyde in CD buffer (1 hr), washed with CD buffer, and dehydrated through a graded acetone series (30%–100%). After critical-point drying (model CPD020, Balzers Union Ltd., Liechtenstein), the disks were sputter coated with gold-palladium alloy (model SCD040, Balzers Union) and viewed with a scanning electron microscope (Hitachi S4000).

For each disk specimen, SEM micrographs (2000×) were taken over three designated fields: one centrally located on the

disk and two near the ends between the scribed lines. The bacterial counts for all three micrographs were enumerated and expressed in bacteria per mm². The mean was obtained from triplicate specimens. For *S. mutans* colonized control specimens, SEM micrographs often demonstrated multilayered bacterial aggregates. An approximation of the bacteria on the surface was made based on the number of bacteria found in a monolayer multiplied by an estimation of the number of layers of bacteria. An ANOVA and Student-Newman-Keuls Multicomparison Tests were used for statistical analysis.

Fluorescence microscopy was used to quantitate the number of *A. naeslundii* on SHA surfaces. A silicon photodetector with digital power meter (models 818SL and 815, Newport Corp., Irvine, CA) was mounted in the camera tube of an optical microscope. A bacteria-coated SHA disk was placed under a 4× objective and illuminated at 490 nm. Light emitted by the fluorescing dye on the bacteria was measured with the photodetector. Disks with no adherent bacteria were used to determine that the average background reading due to light reflection was 40 μW; therefore this value was subtracted out of all subsequent readings of the bacteria-coated disks. The fluorescence of the bacteria on each experimental disk was measured immediately before and after exposure to the Sonicare. The difference in fluorescence was used as an indicator of bacterial removal. Statistical testing of the reduction in photometer readings was completed with an ANOVA along with Student-Newman-Keuls Multicomparison Tests to investigate differences from the control.

Examination of Titanium Surface after Direct Brushing

Six Ti disks, prepared as described above, except without attachment of bacteria, were used to establish the effects of the Sonicare on polished Ti. Two disks were used as controls and received no brushing. Four disks were submerged under 2 mm distilled water and brushed continuously with the Sonicare with direct bristle contact for the equivalent of 3 months normal use (approximating 8 sec brushing per day). The disks were processed for SEM examination. SEM micrographs taken of representative surface areas of both the control and exposed Ti disks (500–20,000 X) were examined for differences in morphology.

Results

Although not shown here, preliminary studies were performed to determine the optimal condition for bacterial colonization of Ti surfaces. Inoculation of *S. mutans* onto Ti disks of the adherence chamber, in the presence of sucrose-containing BHI medium for 24 hr at 37°C allowed the formation of a firmly attached bacterial mass on the Ti disk (Figure 2A). Bacteria were often observed in multilayers or in clusters and were not removable by rinsing with PBS.

P. gingivalis did not attach firmly to Ti disk surfaces when grown in PG broth up to 72 hr at 37°C under anaerobic conditions (data not shown). By following the method of Wu-Yuan *et al.*,[7] it was possible to obtain a Ti surface uniformly covered with *P. gingivalis* as revealed by SEM (Figure 2E). This allowed the enumeration of adherent bacteria on both control and exposed Ti surfaces.

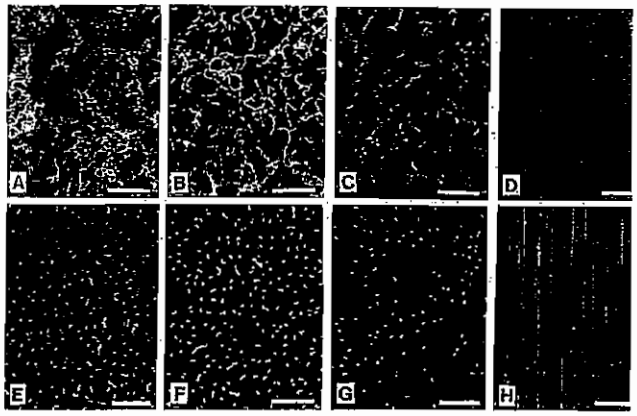

Figure 2. *Bacteria-coated Ti surfaces before and after exposure to Sonicare: S. mutans—panels A, B, C, D; P. gingivalis—panels E, F, G, H. Control surfaces (A&E) exhibit adherent bacteria without exposure to Sonicare. Fewer bacteria remain adherent after 15 seconds of exposure at bristle-to-surface distances of 4 mm (B&F), 2 mm (C&G) and 0 mm (D&H). Bars: 10 μm.*

SEM micrographs taken of the Ti surfaces before and after 15 sec of Sonicare exposure are shown in Figure 2. At a bristle-to-surface distance of 4 mm, there is an obvious reduction in the number of *S. mutans* remaining adherent (Figure 2B), but less so for the *P. gingivalis* (Figure 2F). At a closer distance of 2 mm, there is further removal of *S. mutans* (Figure 2C) and an evident removal of *P. gingivalis* (Figure 2G). When the bristles directly contacted the surface, nearly all the *S. mutans* were removed (Figure 2D) while no adherent *P. gingivalis* were noted (Figure 2H).

The numbers of bacteria adherent to the Ti disks before and after exposure to Sonicare were enumerated and are presented in Table I. The mean value presented in the table represents the average of 9 counts (3 counts of 3 disks for each condition). The control condition, i.e., no Sonicare exposure, is given in the first row of the table with subsequent rows representing increasing proximity of the Sonicare bristles to the disk surface during 15 sec of exposure. A one-way ANOVA indicated a significant difference ($p = 0.0008$ for *S. mutans*, $p = 0.0002$ for *P. gingivalis*) for the exposure conditions. Analysis with *post hoc* t-tests demonstrated a significant reduction ($p < 0.05$) from the unexposed control for all conditions except *P. gingivalis* at 4 mm.

Table I
Dislodgment of *S. mutans* and *P. gingivalis* from Ti surfaces

Exposure (15 sec)	*S. mutans*, 10^3 cells/mm² mean[a] ± se	*P. gingivalis*, 10^3 cells/mm² mean[a] ± se
Control	1860 ± 390	154 ± 19
4 mm	729 ± 36[b]	149 ± 17
2 mm	300 ± 39[b]	60 ± 15[b]
0 mm	7 ± 5[b]	0 ± 0[b]

[a]Mean value obtained from 9 counts (3 counts each of triplicate disks).
[b]Significantly different from control ($p < 0.05$).

Photometric readings of the light emission of FITC-labeled *A. naeslundii* provided a rapid and repeatable method of determining the quantity of bacteria adherent to the SHA disks. The average photometric reading of bacterial-coated disks prior to Sonicare exposure was 383 μW with a standard error of 7.5 μW.

The control conditions, in which the disks underwent handling and placement below the inactivated Sonicare, resulted in a 4.0 ± 0.5% reduction in fluorescence. Table II shows the reduction in adherent bacteria as calculated from the change in photometric readings and with the control reduction subtracted out. As the values in the table demonstrate, a greater distance between the bristles and the surface resulted in the removal of fewer bacteria. Additionally, slightly fewer bacteria were removed with a shorter exposure duration, however the majority of the bacteria that were to be removed were done so within the first 5 sec of exposure. A significant difference ($p < 0.0001$) for the exposure conditions was shown with a one-way ANOVA. *Post hoc* t-tests indicated all reductions were significantly different ($p < 0.05$) than the unexposed control, except for 5 sec at 4 mm.

Table II
Dislodgment of *A. naeslundii* from SHA surfaces

Exposure Condition	Percentage Reduction in Bacteria (mean[a] ± se)		
	5 sec	15 sec	30 sec
1 mm	83 ± 3[b]	87 ± 2[b]	90 ± 1[b]
2 mm	74 ± 2[b]	81 ± 1[b]	85 ± 2[b]
3 mm	54 ± 7[b]	79 ± 2[b]	79 ± 2[b]
4 mm	13 ± 5	24 ± 8[b]	18 ± 10[b]

[a]Mean value obtained from 8 SHA surfaces.
[b]Significantly different from control ($p < 0.05$).

Figure 3 graphically demonstrates the percentage reduction of adherent bacteria from dental surfaces after Sonicare exposure based on the data presented in Tables I and II. The reduction in adherent bacteria was obtained by comparing the numbers of bacteria remaining on Ti disks after exposure to numbers present on the non-exposed disks. There was virtually a 100% reduction in adherent bacteria with bristle contact (0 mm). At a bristle-to-surface distance of 2 mm, the reduction in adherent bacteria was 84% for *S. mutans*, 81% for *A. naeslundii* and 61% for *P. gingivalis*. There was greater variation in the results at 4 mm, with reductions of 61% for *S. mutans*, 24% for *A. naeslundii*, and few *P. gingivalis* being dislodged.

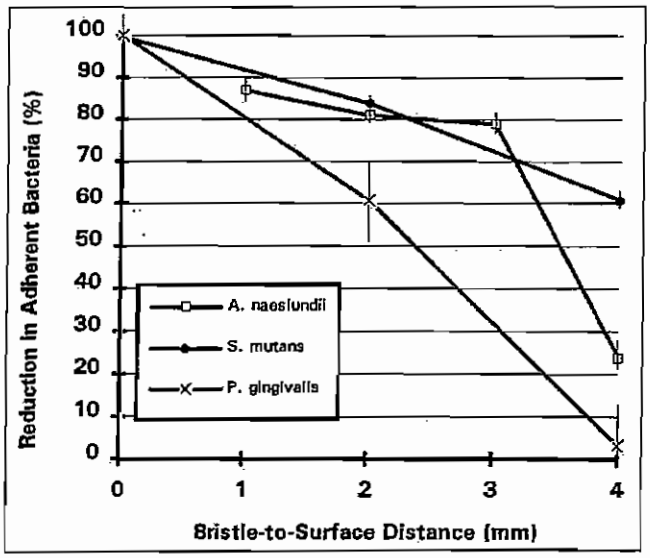

Figure 3. *Reduction in the number of adherent bacteria from model dental surfaces after 15 sec exposure to the Sonicare. The percentage reduction due to Sonicare exposure was calculated as described in the text. Vertical bars indicate the average standard error for each condition.*

Although not presented here, no abrasion of the Ti surface due to direct contact with the Sonicare bristles was revealed when examined with SEM. Both the exposed and non-exposed Ti surfaces, when examined under SEM, appeared smooth with occasional groves created by the polishing procedures.

Discussion

Removal of adherent bacteria associated with dental plaque is important in maintaining the health of the oral cavity. The presence of dental plaque results in tissue irritation that leads to gingivitis, and potentially to periodontal disease or peri-implantitis. Clinical and microbiological findings of healthy and failing dental implants have suggested that bacteria implicated as pathogens in periodontal disease may play a role in the failure of implants.[9-11]

Bacterial adherence and colonization are considered key factors in the etiology of dental plaque and biomaterial-based infections.[12,13] *S. mutans* has been strongly implicated as the etiological agent of dental caries[14] and is involved in early dental plaque formation, as is *A. naeslundii*.[15] *P. gingivalis* is a periodontopathic bacterium that has been associated with periodontal disease.[16] Although the bacteria studied here do not fully represent the complex interbacterial relationships that are involved in dental plaque formation, they are representative of important mechanisms involved in bacterial adherence to dental surfaces.

Many individuals do not adequately remove sufficient dental plaque to maintain healthy teeth and gingival tissue with the use of either manual or conventionally powered toothbrushes. Toothbrushes are limited in their cleaning ability by the requirement of contact between dental plaque and the toothbrush bristles. Although oral irrigators can potentially reach areas toothbrush bristles do not, accurately aiming the water jet on all tooth surfaces is difficult and their efficacy in plaque removal remains doubtful.[1,17] An advantage of the powered toothbrush studied here is that it combines both direct mechanical bristle scrubbing with fluid activity and mild cavitation that extends into areas the bristles do not reach.

The results from this study indicate that the Sonicare creates sufficient fluid activity to remove bacteria without direct bristle contact. Significant bacterial removal was observed at distances up to 4 mm from the bristles (Figure 3). Nearly all adherent bacteria were removed from Ti or SHA surfaces with complete bristle contact or at a distance very close to the exposed surface (Tables I and II). In the oral cavity the greatest buccolingual distance not reachable by toothbrush bristles from either the lingual or buccal side is on average 3 to 4 mm. Removal of bacteria adherent in these areas due to the fluid motion of the Sonicare may compensate for the limitation of contacting all areas with bristles.

The removal of bacteria via dynamic fluid activity at distances beyond 2 mm appears to vary with the characteristics of the adherent bacteria. As evident from Figure 2, exposure of *S. mutans*-colonized Ti disks to Sonicare at 2 and 4 mm reduced

the number of adherent bacteria projecting from, but not directly attached to the surface. This is consistent with the SEM observations of *A. naeslundii* after exposure to sonic vibrations, as previously reported.[4] Bacteria projecting from the surface are at greater risk of being subjected to shear forces from the fluid motion, thus are likely the first to be removed. Bacteria closer and more tightly adherent to the surface may require a longer exposure period or a closer bristle proximity to be effectively removed. In contrast to *S. mutans* which adhered in clusters, *P. gingivalis* appeared to adhere individually to Ti (Figure 2E). It is possible that because the bacteria do not project far beyond the Ti surface, a closer proximity of the bristles to the surface may be required for their removal (Figures 2F and 2G).

In the oral cavity, plaque forms complex interbacterial associations projecting beyond the dental surfaces. With regular Sonicare use, bacteria protruding from a surface may be removed in sufficient quantities to prevent significant additional bacterial accumulation. However, the dynamics of brushing in the oral cavity will be different than an *in vitro* situation, i.e., saliva and toothpaste may alter the fluid dynamics, fluid quantities around the brush head may vary, and bacterial inter-relationships and adherence may be more complex.

In summary, the studies presented here have shown that shear forces associated with mild cavitation and fluid activity surrounding the Sonicare head as it oscillates at 260 Hz are sufficient to remove bacteria adherent to model dental surfaces. Such activity, when combined with direct mechanical scrubbing of the high frequency Sonicare bristle movement, may effectively remove bacteria both on oral surfaces such as teeth and implants and in hard to reach regions of the oral cavity.

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Exhibit O

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Salivary flow and oral complaints in adult dental patients

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Abstract – Saliva plays an important role in maintaining oral health and functions. In the present study, unstimulated and stimulated whole salivary flow and various oral complaints were surveyed in 1427 individuals, 669 men (47%) and 758 women (53%). These individuals, aged 20 to 69 years and from different socioeconomic backgrounds, were recruited from 2000 randomly selected men and women in the register of the public dental health service in northern Sweden. The unstimulated salivary flow rate ranged from 0 to 2.07 mL/min (mean 0.33±SD 0.26) for men, and from 0 to 1.35 mL/min (mean 0.26±SD 0.21) for women. The stimulated salivary flow rate ranged from 0.17 to 7.3 mL/min (mean 2.50±SD 1.06) for men, and from 0 to 6.40 mL/min (mean 2.02±SD 0.93) for women. Women over 55 years of age had a reduced unstimulated salivary flow ($P<0.05$). Individuals with many teeth had a higher stimulated salivary flow than those with fewer teeth ($P<0.001$). Male smokers had a lower unstimulated salivary flow than male non-smokers ($P<0.05$). Women with oral lesion complaints had a lower unstimulated salivary flow ($P<0.05$), and women with burning mouth had a lower stimulated salivary flow ($P<0.01$). Individuals with subjective oral dryness had reduced salivary flow, both unstimulated (men $P<0.01$, women $P<0.001$) and stimulated ($P<0.001$). Subjective oral dryness was associated with complaints of burning mouth ($P<0.001$), muscle pain ($P<0.01$), taste disturbances ($P<0.01$), and dry eyes ($P<0.05$). Individuals with subjective oral dryness had fewer teeth than individuals with no such complaints ($P<0.001$). Information regarding salivary flow rate in adults is important in understanding and evaluating the relationship between salivary flow and various types of oral complaints in patients.

Key words: flow; humans; oral complaints; saliva; stimulated; unstimulated

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There are several studies among selected populations which provide sample information about salivary flow and about individuals who experience dry mouth problems (1–11). A limited number of studies, however, include individuals who are younger than 50 years of age (1, 4, 6, 11). Few studies are population-based, and only one provides information about the prevalence of subjective oral dryness together with data on salivary flow among adults (12). These studies show that there is a wide range of salivary flow rate among individuals, and there are conflicting observations on the relation between age and salivary flow rate.

Saliva plays a crucial role in preserving and maintaining oral health and comfort. Patients with salivary gland hypofunction (13) may complain of subjective oral dryness (14), suffer from eating problems (15), and have difficulties with speech and swallowing (4). The prevalence of dental caries may increase (16), as will opportunistic infections in the oral cavity (17).

Reduced salivary flow and increased subjective oral dryness may be caused by many factors. Numerous medications are associated with salivary gland hypofunction, but also many systemic diseases, such as Sjögren's syndrome (18–23). When examining a patient it can be difficult to decide whether a reduced salivary flow contributes to oral complaints, since very low salivary flow is compatible with excellent oral health in some individuals (24).

The aim of the present cross-sectional study was to determine unstimulated and stimulated salivary flow rates in a non-hospitalized population, aged

20 to 69 years, and to analyze the association of salivary flow with age, gender, and various oral complaints, such as subjective oral dryness, taste disturbances, oral lesions, and burning mouth.

Material and methods

Study population

In 1995 a total of 1000 men and 1000 women were selected at random from the annually updated public dental health service registers of 14 clinics with 48 500 eligible patients. Thus, 10 groups of 100 men and 100 women were selected in each of the 5-year age groups within the age range 20 to 69 years (Table 1).

The clinics were situated in coastal (predominantly urban) and inland/mountain (predominantly rural) areas in the counties of Västernorrland and Västerbotten in northern Sweden. The counties contain half a million inhabitants within an area the size of England & Wales. In northern Sweden, approximately 90% of the population receive regular dental care, and this is provided in approximately equal proportions by the public dental health service and private practitioners (25).

A description of the study, together with an appointment time between 9 am and 11 am, was mailed to each selected individual. If the individual did not attend the first appointment, one reminder was sent. All participants gave their informed consent to being included in the study. The study was approved by the Ethics Committee for Human Experiments at Umeå University.

A total of 1427 individual (669 men and 758 women) from the selected 2000 (70.5%) participated in the study. The age and gender of the participants are presented in Table 1.

The participation rate was similar at all 14 clinics. Out of the 573 non-participants, all but 69 could be contacted by telephone and agreed to have a short interview. Among the 504 interviews, the following reasons for non-attendance were given: unwillingness to participate (44.7%), moved from the area (33.1%), no time (10.3%), too ill to attend (9.3%), or unable to read or understand the language in the communication (2.6%). As can be seen in Table 1, women had a slightly higher attendance rate than men, and for both genders the participation rate

Table 1. Number of participants and some characteristics of the population studied

Age	Number of participants*	Dentate percentage	Number of teeth Mean SD	Medical status†		Tobacco use†	
				Disease percentage	Medication‡ percentage	Smoking percentage	Snuff-taking percentage
Men	669						
20–24 years	44	100	29.0±1.7	2	0	9	34
25–29 years	46	100	29.5±2.2	7	0	7	22
30–34 years	53	100	29.2±2.3	26	8	17	30
35–39 years	67	100	29.1±1.8	14	6	19	22
40–44 years	68	100	27.9±3.5	24	18	13	31
45–49 years	71	99	26.4±5.7	23	20	21	23
50–54 years	80	100	25.0±5.5	23	20	23	16
55–59 years	75	97	21.5±7.6	31	28	17	8
60–64 years	87	93	18.0±9.1	57	56	21	9
65–69 years	78	90	15.3±8.8	58	58	5	9
Women	758						
20–24 years	48	100	28.9±2.1	4	31	21	6
25–29 years	62	100	29.5±2.0	20	28	10	8
30–34 years	70	100	28.8±2.2	29	19	23	6
35–39 years	87	100	28.4±2.4	24	20	31	2
40–44 years	77	100	28.2±2.0	29	22	32	3
45–49 years	84	100	26.4±4.1	21	23	18	0
50–54 years	79	99	25.1±5.3	45	56	32	0
55–59 years	79	100	21.4±6.0	42	51	15	0
60–64 years	82	90	16.6±9.1	47	56	16	0
65–69 years	90	92	16.7±7.8	56	63	9	0

* Equals percentage since 100 participants were invited per group.
† Self-reported.
‡ Hormones included.

increased with age from slightly less than 50% to more than 80%. Because of missing data, the number of individuals reported in the results might be lower than the numbers of individuals given in Table 1.

Determination of salivary flow rate

Prior to the start of the survey, nine dental hygienists and two dentists, each responsible for one or two of the clinics, were given a 1-day program on theoretical and practical issues related to the study. This also included hands-on training and calibration. The author visited all clinics prior to the start of the study and had weekly contact by telephone with all personnel involved.

The letter of invitation to the potential participants included information about the sampling procedure. The individual was also requested to refrain from eating, drinking, toothbrushing and using tobacco for at least 1 h before the appointment. Participants with acute illness were urged to reschedule their appointments. Thus, no test was performed during acute illness. Unstimulated saliva was collected when the participant was positioned in a relaxed position leaning slightly forward. After clearing the mouth by swallowing, saliva was drooled for 10 min into a glass centrifuge tube graded in 0.1-mL increments up to 10 mL (KEBO lab AB, Spånga, Sweden). In rare cases the collection time was reduced or extended (range 5–15 min). Thereafter, the participant was given a standardized piece of paraffin (1 g, melting point 39°C), which was chewed to softness. During this period saliva was swallowed. Then all the stimulated saliva produced was delivered into a graduated glass centrifuge tube for 3 min. In some cases the collection time was reduced or extended (range 1.5–5 min.). Removable dentures were worn during the sampling procedure.

Evaluation of dental status

The number of natural teeth and the presence of fixed and/or removable dentures were recorded by intraoral inspection.

The self-administered questionnaire

A self-administered questionnaire was handed out after saliva sampling. The questionnaire included diagnosed diseases, presence of any acute illness, regularly prescribed medication or over-the-counter medication. Participants were also asked to list any drugs taken during the last 24 h and any periodical intake of medication. The answers were coded yes/no, and the name of the disease/diseases and medication/medications were listed.

The question "Does your mouth usually feel dry?" was used as an indicator of subjective oral dryness. Questions were also asked about other oral complaints, such as oral lesions, taste disturbances and burning mouth. Information about symptoms associated with probable or incipient Sjögren's syndrome, such as eye dryness and muscle or joint pain using the Copenhagen criteria (26) was specifically requested. Regular use of snuff and/or smoking were also assessed.

Furthermore, the level of depression, anxiety and perceived stress were assessed by using the Beck Depression Inventory (BDI) (27), the State and Trait Anxiety Inventory (STAI) (28) and the General Perceived Stress Questionnaire (PSQ) (29). The results from the BDI, STAI and PSQ are to be published or have been published elsewhere (30).

On the basis of the answers in the questionnaire, each participant was then interviewed by the specially trained dental personnel regarding current diseases and ongoing medication. For the evaluation of the data, systemic diseases were classified according to ICD-10 (International Statistical Classification of Diseases and Related Health Problems) (31). Medication was classified according to the WHO guidelines for ATC (Anatomical-Therapeutical-Chemical) (32) and according to a guide for xerogenic medications (22). Healthy participants were defined as individuals without any kind of medication or reported diseases.

Statistical methods

Mean values for salivary flow rate and standard deviation are presented for men and women for the different age groups. Variations between means were tested using the *t*-test. Differences in distribution (proportions) were tested with the chi-square test.

Multiple linear regression with backward elimination was applied with unstimulated and stimulated salivary flow rates as dependent variables and various complaints as independent variables. The regression models were estimated using the weighted least-squares method because of non-constant variance. Data were separated by gender and controlled for age. In order to test various complaints as risk factors, multiple logistic regression with backward elimination was used to test subjective oral dryness as the dependent variable and the same risk factors as above as independent variables. Data were controlled for age and gender. All

tests were two-sided and *P*-values of less than 0.05 were considered to be significant. Statistical routines from the SPSS for Windows (version 7.5) were used.

Results

Salivary flow rates

The unstimulated salivary flow rate ranged from 0 to 2.07 mL/min (mean 0.33±0.26 SD) for men and from 0 to 1.35 mL/min (mean 0.26±0.21 SD) for women. The stimulated salivary flow ranged from 0.17 to 7.3 mL/min (mean 2.50±1.06 SD) for men and from 0 to 6.40 mL/min (mean 2.02±0.93 SD) for women. Both unstimulated and stimulated salivary flow rates were significantly higher in men than in women ($P<0.001$). The unstimulated salivary flow was significantly lower for women over 55 years of age than for younger women ($P<0.05$). The flow of stimulated saliva did not differ significantly among the age groups. There was no unstimulated salivary flow (0 mL/min) in 32 participants, whereas after stimulation by chewing, only one participant had no salivary flow.

Of the 1427 participants, 569 individuals (40%) reported a diagnosed disease or that they were on regular medication, including women taking hormones ($n=71$). All individuals ($n=9$) with a stimulated salivary flow below 0.4 mL/min were diseased and/or were on medication, and so were 19 of the 32 with no unstimulated salivary flow.

Salivary flow in relation to various conditions

The unstimulated and stimulated salivary flow rates (mean, SD) in healthy individuals and among those with various complaints are given in Table 2. A multiple linear regression model, controlling for age, was used to evaluate the data. An association was found between number of teeth and stimulated salivary flow rate ($P<0.001$, $R^2=0.07$) (Fig. 1). This is also shown in Table 2 by using a cut-off point at fewer than 20 teeth.

Ninety-nine men and 164 women (men 15%, women 22%) had an unstimulated salivary flow

Table 2. Unstimulated and stimulated salivary flow rates in groups of subjects with various reported complaints compared to subjects with no such complaints. Data are controlled for age and separated into male and female. Multiple linear regression is used

			Unstimulated salivary flow rate			Stimulated salivary flow rate		
Groups of subjects	Gender	Subjects	Mean SD	Regression coefficient	*P*-value	Mean SD	Regression coefficient	*P*-value
Subjective dry mouth	Males	100	0.25±0.24	−0.078	$P<0.01$	2.09±1.09	−0.447	$P<0.001$
	Females	214	0.18±0.18	−0.105	$P<0.001$	1.69±0.83	−0.420	$P<0.001$
Taste disturbances	Males	6	0.31±0.36	−0.071	NS	2.45±0.88	0.239	
	Females	27	0.18±0.15	−0.056		1.64±0.68	−0.179	NS
Burning mouth	Males	16	0.27±0.24	0.036	NS	2.38±1.36	0.292	NS
	Females	51	0.17±0.17	−0.032		1.58±0.84	−0.388	$P<0.01$
Oral lesions	Males	38	0.40±0.32	0.077	NS	2.42±0.91	0.014	
	Females	41	0.19±0.13	−0.072	$P<0.05$	1.89±0.81	−0.145	NS
Smoking	Males	103	0.28±0.22	−0.063	$P<0.05$	2.31±0.88	−0.215	
	Females	156	0.25±0.20	−0.023	NS	2.05±0.87	0.019	NS
Snuff-taking	Males	125	0.34±0.26	−0.023		2.67±0.90	0.171	
	Females	17	0.28±0.22	−0.075	NS	2.20±0.71	0.172	NS
Dry eyes	Males	45	0.34±0.29	−0.038		2.30±0.97	−0.259	
	Females	91	0.23±0.21	−0.038	NS	1.96±1.02	0.101	NS
Muscular pain	Males	114	0.33±0.26	0.017		2.56±1.15	0.211	
	Females	229	0.25±0.21	0.017	NS	2.04±1.04	0.189	NS
Joint pain	Males	119	0.28±0.23	0.039		2.40±1.17	−0.017	
	Females	176	0.26±0.23	0.038	NS	1.98±1.02	0.019	NS
<20 teeth	Males	108	0.25±0.22	0.041		2.25±1.14	0.373	$P<0.01$
	Females	114	0.19±0.17	0.030	NS	1.76±0.81	0.292	$P<0.01$
Healthy	Males	441	0.35±0.27			2.54±1.02		
	Females	402	0.27±0.20			2.08±0.94		

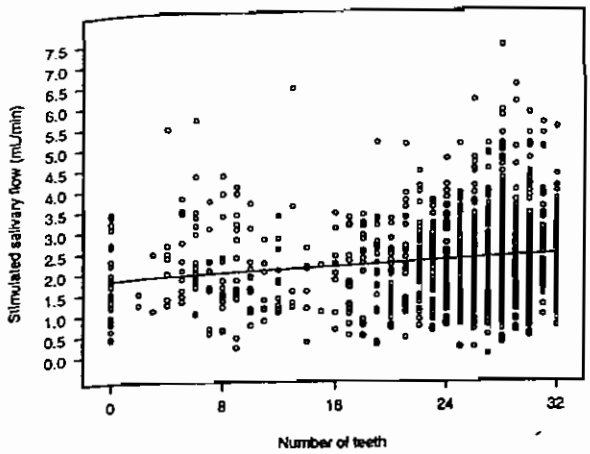

Fig. 1. Stimulated salivary flow in all individuals and the number of teeth in each individual. Regression line indicates the increase in salivary flow with increase in number of teeth ($P<0.001$). The regression model is estimated with weighted least-squares method due to non-constant variance.

below a cut-off level of 0.1 mL/min. Of those, six men and 23 women reported dryness in their eyes and muscular and/or joint pain. One of these 29 participants had previously been diagnosed as having Sjögren's syndrome.

The medications constituted an extensive list with many individuals consuming more than one drug. Men taking xerogenic drugs had a significantly lower unstimulated ($P<0.001$) and stimulated salivary flow ($P<0.01$) and women also had lower unstimulated and stimulated flows ($P<0.05$) than individuals not taking these drugs.

Subjective oral dryness in relation to various conditions

The question "Does your mouth usually feel dry?" was answered "yes" by 22% of the participants (100 men, 214 women). The unstimulated and stimulated salivary flow were significantly lower among these individuals (Table 2). Unstimulated salivary flow less than 0.1 mL/min was found in 35% (28 men, 83 women) of these individuals.

Subjective oral dryness was significantly associated with burning mouth, muscular pain, taste disturbances, dry eyes, and the number of teeth (Table 3).

Medications with known xerogenic effect increased the risk of subjective oral dryness ($P<0.001$).

Discussion

The salivary flow determines the efficacy of the lubricating and cleaning effects of saliva. Unstimulated salivary flow levels were similar to those reported by Heintze and co-workers (33) and by Billing and co-workers (12). However, the stimulated salivary flow rates were higher in the present study than in previous studies on selected samples (1, 2, 4–6, 33), and also higher than the stimulated salivary flow rate reported by Billing and co-workers (12). This might be due to the greater length of the time for collection of the stimulated sample. Another possible explanation might be that this study was conducted in the winter months, meaning that the subjects may have been less dehydrated at that time of the year.

The differences in salivary flow between the genders are confirmed in this study (33), but the physiological importance of this difference is intriguing since it may not mirror a difference between the genders in oral clearance. Lagerlöf & Dawes (34) have shown that oral clearance of well-dispersed substances such as sugar is determined by the salivary flow and by the amount of saliva in the mouth before and after swallowing. These researchers found that the mean amount of saliva in the mouth before swallowing (VMAX) was 1.19±0.39 mL in men and 0.96±0.37 mL in women, and the mean amount of saliva left in the mouth after swallowing (RESID) was significantly higher in men (0.87±0.25

Table 3. Subjective oral dryness (total number=314) in relation to various complaints. Data are controlled for age and gender. Multiple logistic regression is used

Subjects (total number in the study within parenthesis)	Subjects with dry mouth	*P*-value	Odds ratio	Odds ratio (95% CI)
Burning mouth (69)	44	$P<0.001$	5.30	2.88–9.73
Muscular pain (349)	124	$P<0.01$	1.65	1.20–2.27
Taste disturbances (33)	22	$P<0.01$	3.79	1.49–9.67
Dry eyes (138)	59	$P<0.05$	1.65	1.04–2.62
Number of teeth		$P<0.001$	0.97	0.95–0.98

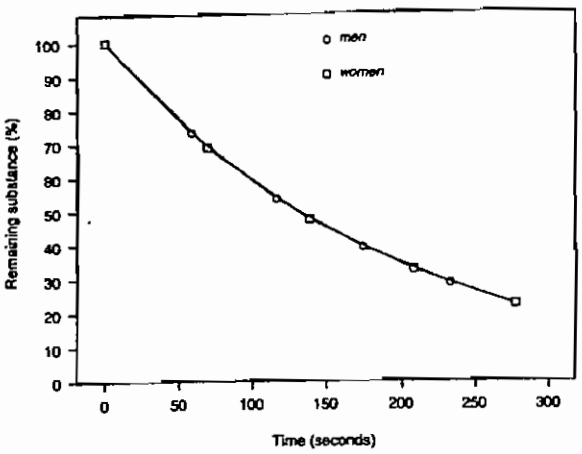

Fig. 2. Clearance time in relation to remaining substances (%) in the mouth of the "average" man and woman. The following parameters were used from the present study: unstimulated salivary flow 0.33 mL/min (men) and 0.26 mL/min (women). From Lagerlöf & Dawes (1984), volume of saliva before swallowing, 1.19 mL (men) and 0.96 mL (women) and volume of saliva after swallowing 0.87 mL (men) and 0.66 mL (women).

mL) than in women (0.66±0.14 mL) (34). These volumes were used together with the mean unstimulated salivary flow rate (men 0.33 mL/min; women 0.26 mL/min) determined in the present study to calculate oral clearance. The time for the men and women to reach VMAX after first swallowing (volume swallowed=VMAX-RESID) was calculated (Fig. 2), after which the concentrations of the well-dispersed substance was calculated. Dilution by newly secreted saliva followed by swallowing continuously eliminates the substance from the mouth (Fig. 2). Thus, it was found that the rate of oral clearance will in fact be the same in men and women despite the difference in salivary flow. When treating patients with caries, special attention should be paid to those with an unstimulated salivary flow lower than 0.1 mL/min (35). In the present study 15% of the men and 22% of the women fulfilled this criterion. However, it is likely that some of these individuals may not in fact have an increased caries risk due to the low salivary flow. They may actually have a salivary flow rate compatible with health.

The previous study supports earlier studies which show that salivary flow rate does not decrease with age in men (36, 37) and is only slightly reduced in women after the menopause (36, 38). This is in contrast to other reports which associate aging with decreased salivary flow (1, 21). In the age group 60 to 69, 25% of the men and 34% of the women had unstimulated salivary flows below 0.1 mL/min. More than half of these individuals had systemic disease and/or intake of medication. The true proportion may even be higher, since some individuals may have reported that they were healthy since they felt healthy although they may have had a disease. In this study no disease or medication was confirmed by medical examination.

In 1933 Sjögren reported that a disorder affecting exocrine glands, including the salivary and lachrymal glands, had been found in 0.05% of the population (39). This is in agreement with the prevalence of diagnosed cases of Sjögren's syndrome in the present study. The results of this study, based on the response to the questionnaire and an unstimulated salivary flow rate of less than 0.1 mL/min, indicate a 2% prevalence of probable or incipient Sjögren's syndrome. It should be emphasized that fulfillment of the criteria of the disease only gives the prevalence of potential cases of the disease. The diagnosis of Sjögren's syndrome must be confirmed by histological examination of salivary gland tissue, serological examination and tests of lacrimal flow (40).

The present study demonstrates the importance of salivary flow in maintaining oral comfort, as individuals with oral complaints were more common among those with low salivary flow.

The representativeness of the population sample may be questioned because individuals not receiving regular dental care were not part of the study. However, in an epidemiological study of 900 randomly selected 35-, 50- and 65-year-old men and women in Västerbotten, 90% of the individuals received regular dental care, and in the age groups 50 years of age and below this was 95% (25).

The low participation rate (approximately 50%) in the youngest age group raises the question of selection bias for this age group. This suspicion is supported by the fact that 20–24-year-old men and women had slightly lower salivary flows than the higher age groups, and that they had slightly fewer teeth than in the higher age groups. There may thus be selection bias in the 20–24-year-old age groups, as the salivary flow in these groups would be expected to be similar to that seen in the higher age groups. Among men below middle age a high percentage were snuff users, a finding confirmed in other studies (41).

The association between salivary flow and various complaints was based on the measurement of salivary flow on one occasion and a follow-up in-

terview about oral complaints in each individual. Although a large number of individuals were studied, the cross-sectional study design precludes causal inferences. However, these findings may be a valuable basis for testing specific causal hypotheses.

Acknowledgments

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Exhibit P

ultreo

Toothbrush-Induced In Vitro Wear of Class V Restorative Materials

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Print This Abstract

Objective

To compare the Ultreo to two controls (a manual and a power toothbrush) after a simulated 1-year typical brushing period with respect to wear/damage to the natural tooth surfaces, cements and restorative materials, and loss/damage to marginal integrity or cement in Class V fillings prepared at the cemento-enamel junction.

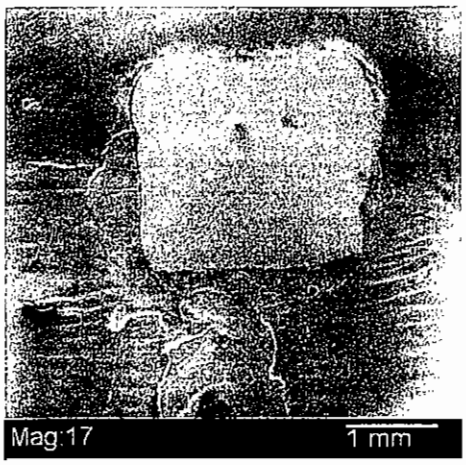
Methods

Human molars were embedded with epoxy in metal specimen holders with the facial surface exposed. Teeth were prepared with cavities measuring 4 x 4 mm centered on the facial cementoenamel junction (CEJ). Five groups of 12 specimens were restored with fillings/indirect restorations of 1) amalgam, 2) nanofilled composite resin, 3) glass ionomer, 4) cast gold-cemented with glass ionomer, 5) pressed ceramic- adhesively cemented with a composite resin cement. The specimens were exposed to the equivalent of one year of brushing using a machine that simulated typical movement of a toothbrush across the specimen under controlled load and toothpaste slurry fluid conditions. Brushing was done with either a manual toothbrush (Oral-B 35) at 250g load or one of 2 power toothbrushes: Braun Oral-B Triumph and Ultreo, both at 125g load. Control specimens remained unbrushed. A qualitative analysis of post-brushed specimens was performed in a scanning electron microscope (SEM). A standardized routine of visual evaluation was followed starting with: 1) low magnification view of the entire tooth, 2) higher magnification examination of tooth root & crown surfaces, restorative surfaces, 3) high magnification of restorative margins & cement. Digital images were captured and viewed on a computer for comparison of the various toothbrush groups and data summarized.

Results

The manual toothbrush consistently had bristle furrows on cementum/dentin root surfaces especially at the heights of contour. The two power toothbrushes had no signs of root surface wear. The manual toothbrush also caused light bristle grooves on the composite resin surfaces. None of the toothbrushes demonstrated breakdown of the restorative margins, any loss of cement, or any effect upon the enamel.

Figure: Representative image of a gold Class V restoration at the CEJ allowing for the examination of adverse effects or wear to the enamel (top), root surface (bottom), the restoration (center) and the adhesive (surrounding the restoration).



Conclusions

- Ultreo was found to be safe on natural tooth surfaces and restorative materials.
- After 1 year of simulated tooth brushing, the manual toothbrush indicated some wear to the root surfaces and some slight wear to the composite resin fillings.
- Neither of the power toothbrushes caused wear of the tooth surfaces or damage to the restorative materials.

This research was supported by National Institutes of Health grant 2R44 DE016761-02.

Back to Ultreo Research

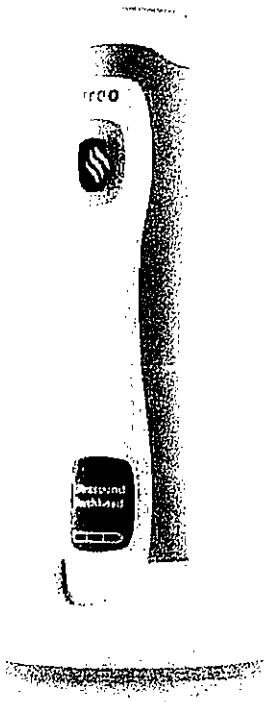


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Exhibit Q

Evaluation of Orthodontic Bracket and Crown Retention after Extended Brushing

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Print This Abstract

Objective

To compare the Ultreo to two controls (a manual and a power toothbrush) after a simulated 2-year typical brushing period with respect to retention force of orthodontic brackets and crowns.

Methods

Standard orthodontic brackets were cemented onto the buccal surfaces of teeth (n=33) using established procedures and materials. Simulated crown preparations (n=32) were created using identical metal dies. The dies simulated a premolar tooth and were fabricated to attach to the base of a tensile force testing machine. Metal castings were fabricated to fit the dies. The castings were cemented to the dies using zinc phosphate cement. Both orthodontic bracket and crown specimens were exposed to the equivalent of two years of brushing using a machine that simulated typical movement of a toothbrush across the specimen under controlled load and toothpaste slurry fluid conditions. Specimens were randomized to treatment. Treatment groups included the experimental group (Ultreo, 125 g load) and two positive controls: a manual toothbrush (Oral-B 35, 250 g load) and a power toothbrush (Oral-B Triumph, 125 g load). Additionally, a negative control with no treatment was included. Subsequent to visual examination, the retention force required to remove the bracket or crown was measured. Bracket and crown retention was determined through the use of shear and tensile testing, respectively. The maximum force at failure (dislodgement of the bracket or crown) was recorded.

Results

The average orthodontic bracket retention force (shear) was calculated for each treatment and is graphically presented in Figure 1. No significant treatment effect upon the orthodontic bracket retention force was found (ANOVA, p>0.05). The average crown retention force (tensile) was calculated for each treatment and is graphically presented in Figure 2. No significant treatment effect upon the crown retention force was found (ANOVA, p>0.05).

Figure 1: Orthodontic Brackets

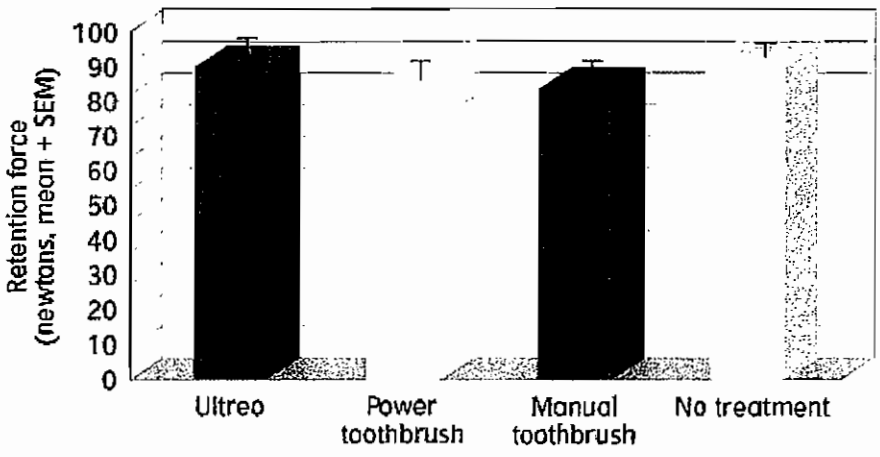
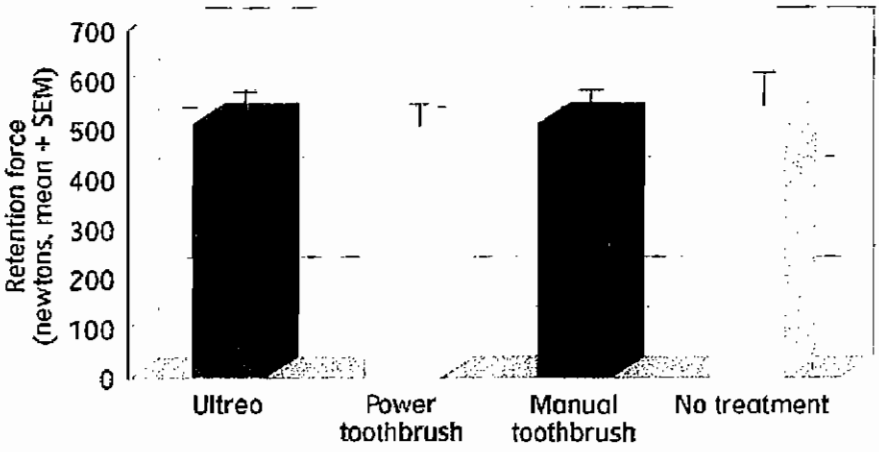


Figure 2: Crowns



Conclusions

- None of the treatments was found to significantly affect the retention force of orthodontic brackets.
- None of the treatments was found to significantly affect the retention force of crowns.

This research was supported by National Institutes of Health grant 2R44 DE016761-02.

Back to Ultreo Research

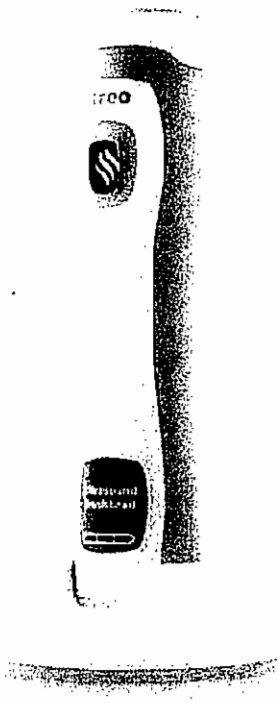


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Exhibit R

Evaluation of the Safety of Sonic and Ultrasound Processes

Roberts FA, Hacker, B, Oswald T
University of Washington, Seattle, WA

Print This Abstract

Objective

The objective of this study was to evaluate the safety of Ultreo's sonic and ultrasound processes using in vitro models of soft tissue.

Methods

Two independent methods were utilized, one to evaluate short term exposure to sonic and ultrasound processes and one to evaluate long term exposure to ultrasound processes. 1) Short term: Human oral keratinocytes (HOK) derived from gingival epithelium were chosen for the soft tissue model. A prototype Ultreo unit with an ultrasound waveguide and independent control over the sonic and ultrasound processes was used to apply treatment to the cells attached to a glass slide. Treatment included exposure (5 s) of the cells to sonic bristle motion wherein the bristles contacted the cells (control) and the following experimental treatments wherein the bristles did not contact the cells (3-4 mm distance): a) sonic bristle motion only, b) ultrasound only, and c) sonic and ultrasound processes synergistically. After exposure the supernatant was evaluated for damaged cells by a lactate dehydrogenase (LDH) assay with the results compared to a known standard curve. LDH is a cytoplasmic enzyme that readily "leaks" from cells when their cell membranes are damaged. 2) Long term: The long-term effect of ultrasound exposure on non-human mammalian cells was assessed with an assay (Stratagene Corp.) that used a target gene which could be screened for DNA damage. Cells were subjected to treatment: (a) negative control with no exposure, (b) positive control with acrylamide (a known mutagen), or (c) ultrasound exposure equivalent to 900 s exposure (3 times expected in vivo exposure). A special ultrasound prototype was used to generate the ultrasound which was coupled into the well holding the cells. After exposure the DNA was harvested and the target gene isolated and evaluated for potential DNA damage via an E. coli host cell and compared to controls.

Results

Two independent methods were utilized, one to evaluate short-term exposure to sonic and ultrasound processes and one to evaluate long-term exposure to ultrasound processes.

1) Short Term: The three experimental treatments, including the synergistic combination of sonic and ultrasound processes with the Ultreo prototype, did not cause significant damage of oral keratinocytes cell membranes with compared with the effect of bristles contact cells ($p>0.05$, see Fig. 1).

2) Long term: In two separate experiments there was no significant difference ($p>0.05$) between the ultrasound treated cells and the negative control cells (see Fig. 2). However, there was a significant difference ($p<0.05$) between the positive control cells (acrylamide) and its control.

Figure 1: Short-term Exposure

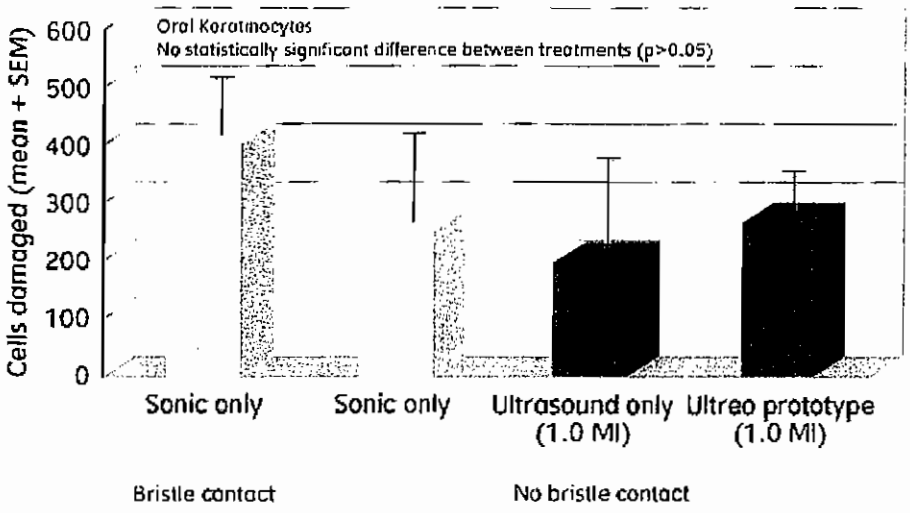
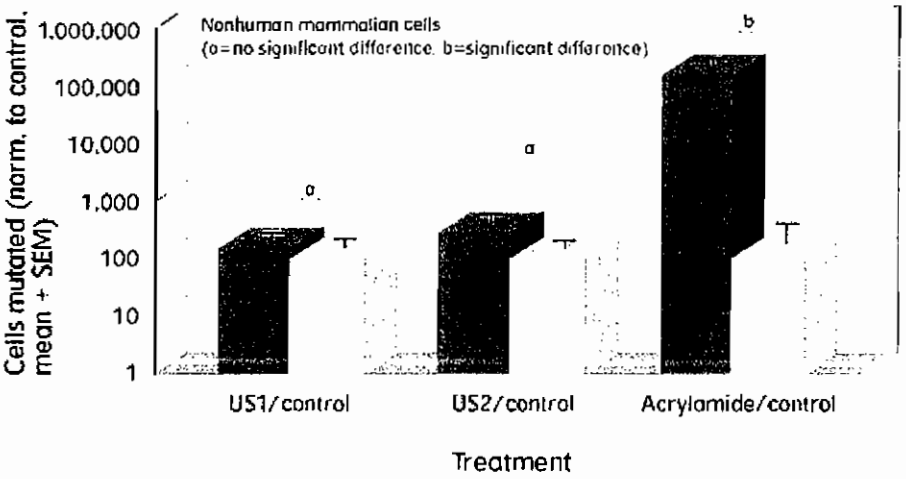


Figure 2: Long-term Exposure



Conclusions

- The sonic and ultrasound processes as found in Ultreo exhibited no adverse effects compared to controls.

This research was supported in part by National Institutes of Health grant 2R44 DE016761-02.

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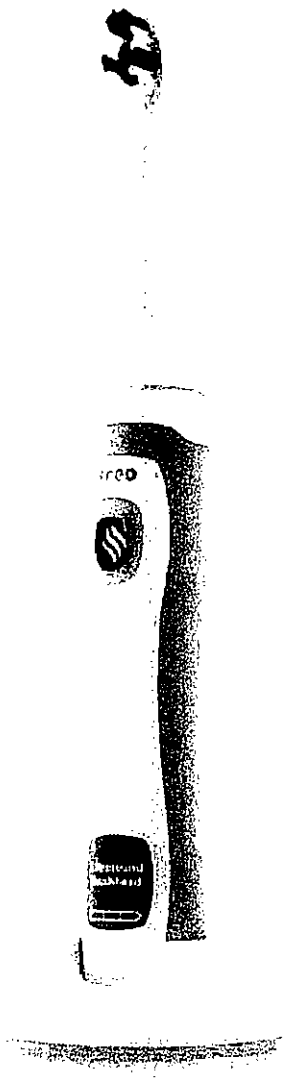

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Exhibit V

CURRICULUM VITAE

November 2007

James Christopher McInnes, Ph.D.

PERSONAL HISTORY

Date of Birth September 28, 1963

Place of Birth Port Angeles, Washington

Citizenship United States of America

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EDUCATION

Institution / Location	Degree	Date	Degree Major
University of Washington Seattle WA	Ph.D.	1992	Bioengineering
University of Washington Seattle WA	BSME	1986	Mechanical Engineering

SERVICE, HONORS, & MEMBERSHIPS

- ANSI ISO TC106/SC7/WG1-4 Dentistry, Normative Standard Development Member (1998-2004), United States expert for powered toothbrushes (2004)
- Member International Association of Dental Research; Oral Health Research Group
- Member Ultrasonic Industry Association
- Tau Beta Pi (Engineering 1984), Pi Tau Sigma (Mechanical Engineering 1985)
- Graduated Cum Laude from University of Washington;

PROFESSIONAL EXPERIENCE

Ultreo, Inc., Redmond, WA (2004 - Present)
Principal Scientist. Oversight and execution of core oral biology research to support concept refinement leading to product development and introduction. Providing bioengineering expertise with respect to efficacy and safety of an oral consumer healthcare device.

Philips Oral Healthcare Inc., Snoqualmie, WA (2000-2004)
Principal Scientist. Coordination of internal and external research. Working with multidisciplinary teams to meet the clinical testing needs of product innovation, development and marketing. Analysis of new techniques and methods to quantitate efficacy and safety of oral hygiene products.

Shanghai Jiao Tong University, Shanghai, China (1997-1998)
Instructor. Taught English language skills to doctorate, Master of Business Administration and English major students. Focus of work was on technical writing and oral communication for engineering, scientific and business pursuits. Developed own curriculum in a diverse skills environment.

Optiva Corporation, Bellevue, WA (1992-1997, 1998-2000)
Sr. Research Scientist. Work included studying scientific and clinical efficacy of a sonic toothbrush that utilizes sonic vibrations to aid in the removal of oral bacteria associated with plaque. Position included research and development on product improvements and new dental devices.

Research Center in Oral Biology, University of Washington (1987-1992)
NIDR Pre-Doctoral Fellowship. Investigated sonic vibrations as a means to disperse and/or damage oral bacteria in the oral cavity. This research project included constructing an apparatus for the generation of sonic vibrations, investigating methods of quantifying low-frequency sonic energy, developing assays for the detection of bacteria adherent to a model tooth surface, and analyzing exposed bacteria for damage via electron microscopy.

PATENTS

US 7,269,873 B2 Ultrasonic toothbrushes employing an acoustic waveguide. Gerald K. Brewer, James Christopher McInnes, Daniel Bayeh, Fredrick Jay Bennett, Richard K. Taylor, David A. Ballard, George A. Barrett, September 18, 2007

US 6,309,835 Methods for quantitating the efficacy of oral care products. Lokanathan M. Iyer, Robert E. Akridge, James C. McInnes, October 30, 2001

US 6,202,241 Brushhead for use in an acoustic toothbrush. Thomas Hassell, Stephen M. Meginness, III; James C. McInnes; March 20, 2001

US 5,784,742 Toothbrush with adaptive load sensor. David Giuliani, Ryan W. McMahon; James Christopher McInnes, July 28, 1998

PRESENTATIONS / LECTURES

- "From Concept to Commercialization: Taking a product from academia to production". 2007 EPI Forum, 2007
- "Incorporating ultrasonics into the electronic toothbrush" Improving Great Products 2 - The SBIR and STTR Programs: A Pathway to Translational Research, International Association for Dental Research, 2007.
- "Sonic Technology & Research" Emerging Trends in Oral Care, Symposium sponsored by Philips Oral Healthcare. 2002, 2003, 2004.
- "Disruption of Dental Plaque Biofilm via Fluid Forces" Montana State University, Center for Biofilm Engineering, TAC Meeting 2003
- "Oral Cavity Hydrodynamics – Dental plaque growth, removal, and the effects of powered brushing." A Cytergy short course. 2003

PUBLICATIONS:

Journals

Mourad PD, Roberts FA, McInnes C. Synergistic use of ultrasound and sonic motion for removal of dental plaque bacteria. *Compend Cont Educ Dent* 2007,, 28(7): 354-358.

Yuen A, Nelson R, Johnson MR, McInnes C, Nguyen HK, Sorensen JA,. *In vitro* evaluation of the efficacy and safety of the IntelliClean System: Interproximal biofilm removal and dentin substrate wear. *Compend Cont Educ Dent* 2004, 25 (Suppl 1) 44-50.

McInnes C, Pace J. Designing the next generation of a sonic toothbrush. *Am J Dent* 2002; **15**: 4B-6B.

Wu-Yuan CD, Anderson RD, McInnes C. Ability of the Sonicare electronic toothbrush to generate dynamic fluid activity that removes bacteria. *J Clin Dent* 1994; **5**: 89-93.

Johnson B, McInnes C. Clinical evaluation of the efficacy and safety of a new sonic toothbrush. *J Periodontol* 1994; **65**: 692-697.

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McInnes C, Engel D, Martin RW. Fimbria damage and removal of adherent bacteria after exposure to acoustic energy. *Oral Microbiol Immunol* 1993; **8**: 277-282.

McInnes C, Engel D, Moncla BJ, Martin RW. Reduction in adherence of *Actinomyces viscosus* after exposure to low-frequency acoustic energy. *Oral Microbiol Immunol* 1992; **7**: 171-176.

McInnes C, Engel D, Martin RW. Bacterial luminescence: A new tool for investigating the effects of acoustic energy and cavitation. *J Acoust Soc Am* 1990; **88**: 2527-2532.

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McInnes C, Yuen A, Johnson MJ. In vitro methods to evaluate plaque biofilm removal from interproximal locations. *J Dent Res* 2004; **83** (IADR Abstract #165)

Moritis K, Johnson MR, McInnes C. Investigation of the influence of a powered toothbrush's bristle motion on plaque reduction and safety. *J Dent Res* 2003; **82** (AADR Abstract#1757)

Delaurenti M, Platt K, Johnson MR, McInnes C. Development of a powered toothbrush head for improved efficacy. *J Dent Res* 2003; **82** (AADR Abstract#1758)

McInnes C. Fluid Dynamics. *Prac Rev Ped* Dent 2002; **13**: 5.

McInnes C. Water World – Fluid dynamics in the mouth shape how biofilms grow and survive. Scientific American custom publication: "Emerging Trends in Oral Care – The Biofilm Revolution". 2002

McInnes C, Hill JS, Johnson MR. Effect of toothbrushes on fluid deposition into a model periodontal pocket. *J Dent Res 2001*; **80** (AADR Abstract #668): 119

Hill JS, McInnes C, Johnson MR, Ballard DA. In vitro assay of tooth contact by toothbrushes. *J Dent Res 2001*; **80** (AADR Abstract #669): 119

McInnes C, Moncla B, Engel D, Martin RW. Biological effects of acoustic energy on the oral bacteria *Wolinella recta*. *Proc of the Annu Int Conf IEEE Eng Med Biol Soc* 1989; **11**: 1644-1645.

Dissertation

McInnes JC. Low-frequency acoustic energy, cavitation, and their effects on bacteria. Doctoral Dissertation, University of Washington, 1992.

GRANTS

SBIR Grant #2R44 DE016761-02 "Rapid Plaque Removal by a Sonic and Ultrasonic Toothbrush" Key Personnel (2006)

SBIR Grant #1 R43 DE11639-01 "Pressure Sensing Sonic Toothbrush", Principal Investigator (1996)